TAD. This energy difference will contribute to the difference in enthalpy of binding between the two ligands. The requirements for cofactor binding to IMPd may be similar to those seen in the structures of other dehydrogenases. Thus, the fact that the carboxamide group in TAD is already locked into the bound conformation may explain in part its tighter binding to IMPd with respect to NAD^+.

It should be noted that TAD does bind to several other dehydrogenases with an affinity only comperable to that of NAD^+.[15] This indicates that carboxamide conformation is only one of a number of potential factors which will influence the enzyme binding of TAD. For example, weaker binding of TAD to several dehydrogenases has been attributed to a possible failure of these enzymes to maintain an energetically favored close sulfur–oxygen contact in the bound inhibitor.[15,18] If this interaction were not maintained by a particular binding site, it would be of sufficient magnitude to counter any advantage offered by the favorable carboxamide conformation in these enzymes. It is to be expected that dehydrogenase binding by a complex molecule will be influenced by multiple conformational factors. Nevertheless, constraint of carboxamide group rotation in the thiazole-4-carboxamide moiety will be one of these factors, and will have a significant effect on the binding of TAD to its target enzyme.

Acknowledgment. We thank Dr. Jeffrey P. Jones for helpful discussions and for introducing us to the NBO method. Work supported in part by Public Health Service Grant CA 45145.

Registry No. TAD, 83285-83-0; $NAD(P)^+$, 53-59-8; 4-thiazolecarboxamide, 3575-09-5; 1,4-dihydronicotinamide, 18940-08-4; nicotinamide, 98-92-0; dehydrogenase, 9035-82-9.

Supplementary Material Available: Citations for structures obtained from the Cambridge Structural Database (Table I) and from the Protein Data Bank (Table II) (7 pages). Ordering information is given on any current masthead page.

5,5-Disubstituted Hydantoins: Syntheses and Anti-HIV Activity

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A series of 5,5-disubstituted hydantoin derivatives was synthesized by alkylating 5,5-bis(mercaptomethyl)-2,4-imidazolidinedione (**3**) with various halomethylaromatic or halomethylheteroaromatic precursors, or by using the Buchener–Berg procedure on the required ketone. When evaluated for their ability to inhibit HIV-induced cell killing and virus production in CEM or MT-2 cells only compounds **2**, **4n**, **4o**, and **4i** demonstrated modest activity, the latter with an IC_{50} = 53 μM.

Introduction

Many strategies[1a-e] have been utilized in the design of new chemotherapeutic agents for the treatment of AIDS. Generally, new compounds have been designed to interfere with any of a number of key steps in the replicative cycle of the human immunodeficiency virus (HIV), the causative agent for this life-threatening disease. One strategy that has provided a number of promising compounds has been the disruption of virus adsorption to the host-cell membrane. This interaction is known to rely on an affinity of the virally-encoded glycoprotein gp120 for the cellular CD4 receptor of the host. Compounds that have been shown to interfere with this interaction include soluble forms of CD4, aurintricarboxylic acid, and various sulfated polysaccharides.[1b]

In 1986 Lehr and Zimmer reported that diphenylhydantoin (dilantin, **1**), also a membrane-reactive drug that has been used in antiepileptic therapy for some 40 years, inhibits HIV binding to CD4 positive lymphocytes.[2] More recently, these findings have been extended to suggest that the aforementioned inhibition is likely due to host-cell membrane fluidization resulting in a reduced availability of the CD4 receptor for ligand interaction.[3] As a complement to this work, it has been demonstrated that dilantin suppresses the influx of Ca^{+2} ions that occurs shortly after HIV infection, suggesting a possible role of membrane-associated calcium-dependent cellular processes in HIV infection.[4] For several years we have had an interest in developing anti-AIDS drugs by targeting biological processes associated with the HIV glycoprotein coat. We have synthesized a number of polysaccharides[5] that were designed to interfere with the biosynthesis of gp120.

(1) This area has been reviewed by several authors including (a) De Clercq, E. Basic Approaches to Anti-Retroviral Treatment. *J. Acquired Immune Defic. Syndr.* **1991**, *4*, 207–218. (b) De Clercq, E. Targets and Strategies for the Antiviral Chemotherapy of AIDS. *Trends Pharmacol. Sci.* **1990**, *11*, 198–205. (c) Mitsuya, H.; Yarchoan, R.; Broder, S. Molecular Targets for AIDS Therapy. *Science* **1990**, *249*, 1533–1544. (d) Norbeck, D. W. Recent Advances in Anti-Retroviral Chemotherapy for AIDS. *Annu. Rep. Med. Chem.* **1990**, *25*, 149–158. (e) Hirsch, M. S. Chemotherapy of Human Immunodeficiency Virus Infections: Current Practice and Future Prospects. *J. Infect. Dis.* **1990**, *161*, 845–857.

(2) Lehr, H. A.; Zimmer, J. P. Diphenylhydantoin zur Prophylaxe und Therapie von AIDS. *DMW, Dtsch. Med. Wochenschr.* **1986**, *111*, 1001–1002.

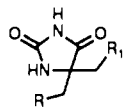
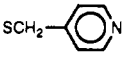
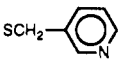
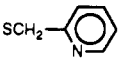
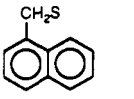
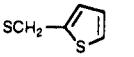
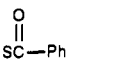
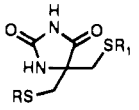
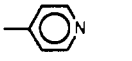
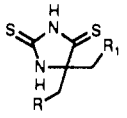
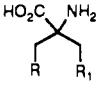
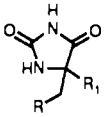
(3) Lehr, H. A.; Zimmer, J. P.; Hübner, C.; Ballmann, M.; Hachmann, W.; Vogel, W.; Baisch, H.; Hartter, P.; Albani, M.; Kohlschütter, A.; Schmitz, H. Decreased Binding of HIV-1 and Vasoactive Intestinal Peptide Following Plasma Membrane Fluidization of $CD4^+$ Cells by Phenytoin. *Virology* **1990**, *179*, 609–617.

(4) Cloyd, M. W.; Lynn, W. S.; Ramsey, K.; Baron, S. Inhibition of Human Immunodeficiency Virus (HIV-1) Infection by Diphenylhydantoin (Dilantin) Implicates Role of Cellular Calcium in Virus Life Cycle. *Virology* **1989**, *173*, 581–590.

(5) Comber, R. N.; Secrist, J. A., III; Schutzbach, J. S.; Manguikian, R. A. Thio-Linked Mannopolysaccharides: Synthesis and Anti-HIV Activity. Manuscript in preparation. (b) Comber, R. N.; Secrist, J. A., III; Dunshee, D. A.; Friedrich, J.; Petty, S. Thio-Linked Glucopolysaccharides: Synthesis and Anti-HIV Activity. Manuscript in preparation.

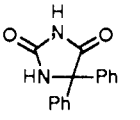
0022-2623/92/1835-3567$03.00/0

Table I. Compounds Studied

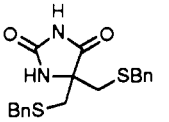
structure	compound no.	substituents R = R_1	antiviral activity (μM) CEM IC_{50}[a]	CEM TC_{25}[b]	MT-2 IC_{50}	MT-2 TC_{25}
	**2**	SCH_2Ph	–[c]	NT[d]	17	NT
	3	SH	0.6	3	–	0.8
	4a	OCH_2Ph	–	>100[e]	–	87
	4b	$SCH_2C_6H_{11}$	–	8	–	12
	4c	$SCH_2C_6H_4$-*p*-Br	–	16	–	7
	4d	$SCH_2C_6H_4$-*p*-OMe	23	6	–	6
	4e	$SCH_2C_6H_4$-*p*-CN	25	15	–	8
	4f	$SCH_2C_6H_4$-*p*-CO_2H	–	NT	–	NT
	4g	$SCH_2C_6H_4$-*p*-CO_2Me	–	25	–	10
	4h	$SCH_2C_6H_4$-*p*-NO_2	19	9	–	5
	4i		53	NT	–	45
	4j		–	NT	–	NT
	4k		–	NT	81	NT
	4l		–	NT	–	NT
	4m		–	NT	–	17
	4n	$SCH_2C_6H_4$-*p*-NH_2	88	NT	6	16
	4o		7.0	31	–	5
	**5a**		–	NT	–	NT
	5b	Ph	–	64	–	50
	**6**	SCH_2Ph	–	16	–	16
	**10**	SCH_2Ph	–	NT	–	60
	**7**	R = SCH_2Ph R_1 = H	–	5	–	2
	8	R = SCH_2Ph R_1 = CH_2SH	–	34	–	50
	9	R = SCH_2Ph R_1 = $CH_2S(CH_2)_2OH$	–	NT	–	NT

[a] IC_{50} = the minimum drug concentration (μM) that inhibited CPE by 50%, calculated by using a regression analysis program for semilog curve fitting. [b] TC_{25} = the minimum drug concentration (μM) that reduced cell viability by 25%. [c] (–) = compound was inactive. [d] NT = nontoxic up to 100 μM. [e] Compound was showing toxicity, however TC_{25} was greater than 100 μM, the highest concentration tested.

Similar approaches have been used by others to obtain such potent anti-HIV compounds as *N*-butyldeoxynojirimycin and related glycosylation inhibitors.[6] Herein we report on a series of hydantoins related to dilantin (1) that we prepared as a result of discovering anti-HIV activity in 5,5-bis[[(phenylmethyl)thio]methyl]-2,4-imidazolidinedione (2), a related compound.



1



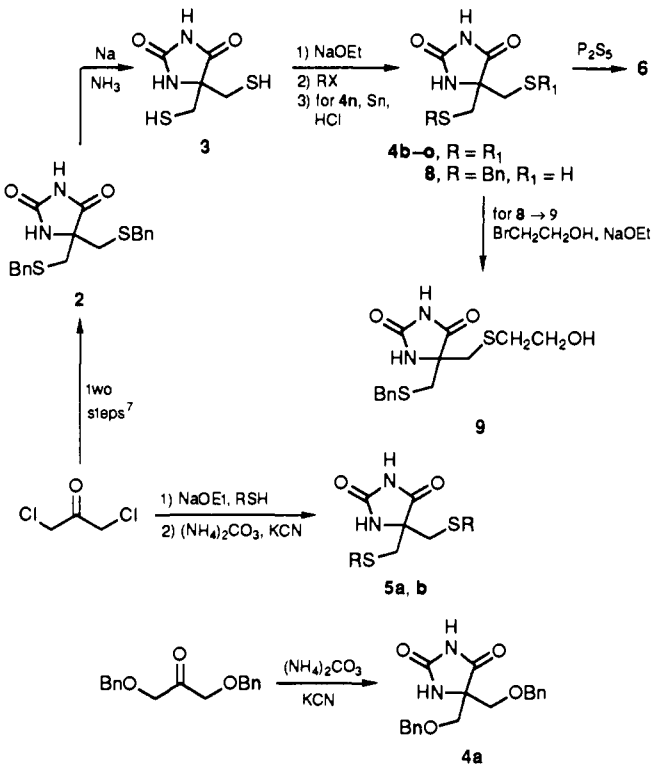
2

Chemistry

The structures of the compounds prepared in this study are shown in Table I. The starting material for the

(6) Fleet, G. W. J.; Karpas, A.; Dwek, R. A.; Fellows, L. E.; Tyms, A. S.; Petursson, S.; Namgoong, S. K.; Ramsden, N. G.; Smith, P. W.; Son, J. C.; Wilson, F.; Witty, D. R.; Jacob, G. S.; Rademacher, T. W. Inhibition of HIV Replication by Amino-Sugar Derivatives. *FEBS Lett.* 1988, *237*, 128–132.

Scheme I



syntheses of most of these targets was 5,5-bis(thiomethyl)-2,4-imidazolidinedione (**3**) (Scheme I),[7] which was readily obtained from the known 5,5-bis[[(phenylmethyl)thio]methyl]-2,4-imidazolidinedione (**2**).[8] The other targets were prepared from the appropriate ketone using the Buchener–Berg procedure.[9] Thus, **2** was converted into the key intermediate **3** by treatment with sodium in liquid ammonia followed by chromatographic purification. For the synthesis of hydantoins **4b–o**, compound **3** was treated with 3 equiv of NaOEt followed by 2 equiv of the required alkylating agents, which were all commercially available with the exception of 2-(chloromethyl)thiophene[14] used in the preparation of **4m**. In all cases the yields were moderate to good, though few attempts were made at yield optimization. Compound **4n** was obtained by reducing the nitro group in **4h** with granular tin in HCl.[15] Compound **2** was treated with P_2S_5 to obtain the 2,4-dithioxohydantoin derivative **6**. Monoalkylated hydantoin **8** was obtained by treating the trianion of **3** dropwise at 0 °C with only 1 equiv of benzyl bromide followed by chromatography to remove any **2** that formed. The unsymmetrical hydantoin **9** was formed by alkylating the dianion of **8** with 2-bromoethanol. The only other unsymmetrical hydantoin, the hydantoin of *S*-benzylcysteine (**7**), was resynthesized according to the literature procedure.[16] Compound **10** was resynthesized by barium hydroxide hydrolysis of **2**.[8]

As noted above, the Buchener–Berg method was used to synthesize several of the targets. Hydantoins **5a** and **5b** were synthesized from 1,3-dichloro-2-propanone by initial displacement reaction with 4-mercaptopyridine or thiophenol, respectively, followed by treatment of the resultant ketones with potassium cyanide and ammonium carbonate. Compound **4a**, in which the side-chain heteroatom is oxygen instead of sulfur, was formed in an analogous fashion from 1,3-bis(benzyloxy)-2-propanone.[17]

Biological Data

The compounds synthesized in this study were tested for their ability to inhibit HIV-induced cell killing and virus production in CEM or MT-2 cells. The latter were added to each well of a 96-well round-bottomed microtiter plate at 5×10^3 cells per wall. The cells were infected with virus at a multiplicity of infection predetermined to give complete cell killing at 6 days postinfection. The multiplicity of infection (MOI) of HIV-1_{RF} utilized in these experiments was 0.01 with CEM cells and 0.005 with MT-2 cells. Serial half-log dilutions, a total of five for each compound, starting from a high test concentration of 100 μM, were added to appropriate wells in triplicate to evaluate their potential to inhibit the virus. Controls for each assay included drug cytotoxicity control (cells + drug), virus control (cells + virus), cell viability control (cells only), and drug colorimetric control (drug only). AZT was run in parallel as a positive control compound. Following 6 days of incubation at 37 °C, the viability of the cells in each well was determined spectrophotometrically according to the XTT method as described by Weislow et al.[18]

As mentioned above, activity was initially detected in compound **2**, which served as a starting point for structure modification. Using the above procedures **2** was found to be active in MT-2 cells (IC_{50} = 17 μM) and was nontoxic at the highest dose tested. Compound **2** was inactive when evaluated in CEM cells. Compounds **3**, **4a–o**, and **5a–10** were all similarly tested. Among the strategies that were incorporated into the design of these analogues were (1) substitution of oxygen for sulfur in the side chains emanating from the hydantoin 5-position (e.g., **4a**); (2) removal of one of the substituents at the 5-position (e.g., **7**); (3) varying the length of these substituents (e.g., **5a,b**); (4) removal of one or both alkyl groups capping sulfur on the side chains (e.g., **3**, **8**); (5) saturation of the aromatic ring

(7) Compound **3** has been synthesized and isolated, but not characterized. Rodgers, T. R.; LaMontagne, M. P.; Markovac, A.; Ash, A. B. Hydantoins as Antitumor Agents. *J. Med. Chem.* **1977**, *20*, 591–594.

(8) Shen, T.-Y.; Walford, G. L. Antiinflammatory 4-Amino-1,2-dithiolane-4-carboxylic Acids. *U.S. Patent* 3,547,948. *Chem. Abstr.* **1971**, *75*, 6336j.

(9) As noted by Rodgers et al. (ref 7), the Buchener–Berg reaction has been reviewed by Ware,[10] and modified by Henze,[11] Spurlock,[12] and Abshire and Berlinguet.[13]

(10) Ware, E. The Chemistry of the Hydantoins. *Chem. Rev.* **1950**, *46*, 403–470.

(11) Henze, H. R.; Speer, R. J. Identification of Carbonyl Compounds Through Conversion into Hydantoins. *J. Am. Chem. Soc.* **1942**, *64*, 522–523. Henze, H. R.; Isbell, A. F. Researches on Substituted 5-Phenylhydantoins. *Ibid.* **1954**, *76*, 4152–4156.

(12) Spurlock, J. J. Hydantoins as Anticonvulsants. I. 5-*R*-5-(2-Thienyl)-hydantoins. *J. Am. Chem. Soc.* **1953**, *75*, 1115–1117.

(13) Abshire, C. J.; Berlinguet, L. Synthesis of α-Alkyl-Substituted Amino Acids and Derivatives. *Can. J. Chem.* **1965**, *43*, 1232–1234.

(14) Wiberg, K. B.; McShane, H. F. 2-Chloromethylthiophene. *Organic Syntheses*; Wiley: New York, 1955; Collect. Vol. III, pp 197–199.

(15) Schönberg, A.; Iskander, Y. Organic Sulfur Compounds. Part XXVII. The Relation Between the Constitution of Thioethers and Thiols and Their Sensitivity Towards Alkali. *J. Chem. Soc.* **1942**, 90–95.

(16) Chimiak, A.; Eisler, K.; Jost, K.; Rudinger, J. Amino Acids and Peptides. LXXX. Unambiguous Syntheses of *N*-Carbamyl-Oxytocin and *N*-Carbamyl-2-*O*-Methyltyrosine-Oxytocin. *Collect. Czech. Chem. Commun.* **1968**, *33*, 2918–2926.

(17) Ogilvie, K. K.; Nguyen-Ba, N.; Hamilton, R. G. A Trihydroxy Acyclonucleoside Series. *Can. J. Chem.* **1984**, *62*, 1622–1627.

(18) Weislow, O. S.; Kiser, R.; Fine, D. L.; Bader, J.; Shoemaker, R. H.; Boyd, M. R. New Soluble-Formazan Assay for HIV-1 Cytopathic Effects: Application to High-Flux Screening of Synthetic and Natural Products for AIDS-Antiviral Activity. *J. Natl. Cancer Inst.* **1989**, *81*, 577–586.

in the side chains (e.g., **4b**); (6) and varying the substituents on, or the nature of, the aromatic ring (e.g., **4c–n**). Very little was done to modify the hydantoin ring except to replace the oxygen atoms with sulfur (6) and to hydrolyze the ring completely to a disubstituted glycine derivative (**10**).

Inspection of Table I reveals that almost all of these manipulations resulted in loss of activity. No compound was very potent at a nontoxic dose. The only compounds that demonstrated activity other than compound **2**, were **4i**, **4n**, and **4o**, which did so only when evaluated in the CEM cell line. Of these, only compound **4i** had a meaningful window of activity (IC_{50} = 52.7 μM and was nontoxic up to the highest dose tested).

The above results suggest that this area warrants modest interest, but before further pursuit of worthwhile compounds can be undertaken, an understanding of the mechanism(s) of action of these compounds must be developed. We have begun that effort, with preliminary results showing that the compounds are not inhibitors of reverse transcriptase (data not shown). A full account of our mechanism of action studies on these hydantoins will be presented in due course.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. NMR spectra (internal Me_4Si) were recorded with a Nicolet NT 300NB spectrometer operating at 300.635 MHz for ^1H and at 75.6 MHz for ^{13}C. Mass spectra were recorded with a Varian MAT 311A mass spectrometer in the FAB or EI mode. Microanalyses were performed by the Molecular Spectroscopy Section of the Organic Chemistry Research Department at Southern Research Institute. The alkylating agents used in the syntheses of compounds **4b–n** (except 4m) were all commercially available. The vendor will be noted in the individual experimentals. Chromatography column sizes are given as width × length.

5,5-Bis(mercaptomethyl)-2,4-imidazolidinedione (3). Compound **2**[8] (55.4 g, 0.15 mol) was placed in liquid ammonia (ca. 400 mL), and fresh sodium pieces were added until a blue endpoint was reached (this reaction requires overhead stirring). After 10 min of a permanent blue coloration, ammonium chloride was added to discharge the blue color. The ammonia was allowed to evaporate overnight under a stream of N_2. The residue was taken up in 200 mL of degassed water, chilled, acidified with concentrated HCl, and evaporated to dryness. The compound was preadsorbed on silica gel and chromatographed (13.5 × 12 cm, silica gel 60, 70–230 mesh) eluting with 97:3 $CHCl_3$–MeOH to give 15.5 g of crude **3**. Trituration with 97:3 $CHCl_3$–MeOH gave (in several crops) 13.26 g (46%) of **3**: mp 192–194 °C; FABMS (M + 1) 193; ^1H NMR (Me_2SO-d_6) δ 2.36 (br s, 2 H, SH), 2.70, 2.79 (AB q, J = 14 Hz, 4 H, $HSCH_2$), 7.77 (br s, 1 H, amide NH), 10.83 (br s, 1 H, imide NH). Anal. ($C_5H_8N_2O_2S_2$) C, H, N.

5,5-Bis[(phenylmethoxy)methyl]-2,4-imidazolidinedione (4a). Compound **4a** was prepared by reacting the symmetrical ketone 1,3-bis(benzyloxy)acetone[17] (2.0 g, 7.4 mmol) with potassium cyanide (0.72 g, 11.1 mmol) and ammonium carbonate (4.4 g, 45.8 mmol) according to the procedures of Shen and Walford.[8] Upon filtration and chromatography of the resulting oil on a 2 × 20 cm column of silica gel, compound **4a** was obtained as a white solid, 1.01 g (40%): mp 73–74 °C; FABMS (M + 1) 341; ^1H NMR (Me_2SO-d_6) δ 3.49, 3.55 (AB q, J = 10 Hz, CH_2-hydantoin), 4.50 (m, 4 H, benzylic CH_2), 7.12–7.42 (m, 10 H, aromatics), 8.12 (br s, 1 H, amide NH), 10.64 (br s, 1 H, imide NH). Anal. ($C_{19}H_{20}N_2O_4$) C, H, N.

General Method for the Preparation of Hydantoins 4b–m. A 0.05–0.1 M solution of the trianion of hydantoin **3** was generated by adding **3** to a deoxygenated solution of NaOEt (3 equivs, prepared from Na and EtOH) in EtOH under nitrogen, and the clear solution was stirred for 15 min before the requisite alkylating agent was added all at once. The reaction mixture was stirred for 24 h with gradual precipitation of reaction salts. Using 1 N HCl the pH was adjusted (as judged by pH paper) to between 7 and 8, salts were filtered off, the filtrate was condensed, and the residue was chromatographed on silica gel 60 (230–400 mesh) with $CHCl_3$–MeOH mixtures as eluant. The products were analyzed as such or crystallized from the indicated solvent(s). In this manner the following compounds were obtained.

5,5-Bis[[(cyclohexylmethyl)thio]methyl]-2,4-imidazolidinedione (4b). Starting materials were 0.2 g (1.04 mmol) of **3** and 0.37 g (2.08 mmol) of (bromomethyl)cyclohexane (Fluka Chemika-BioChemika); after chromatography on a 2 × 20 cm column of silica gel, compound **4b** was obtained as a white solid: 110 mg (26%); mp 210–212 °C; FABMS (M + 1) 385; ^1H NMR (Me_2SO-d_6) δ 0.76–1.80 (complex multiplets, 22 H, cyclohexyl), 2.43 (d, J = 7 Hz, 4 H, CH_2-cyclohexyl), 2.70, 2.79 (AB q, J = 14 Hz, 4 H, CH_2-hydantoin), 7.83 (br s, 1 H, amide NH), 10.72 (br s, 1 H, imide NH). Anal. ($C_{19}H_{32}N_2O_2S_2$) C, H, N.

5,5-Bis[[[(p-bromophenyl)methyl]thio]methyl]-2,4-imidazolidinedione (4c). Starting materials were 0.3 g (1.56 mmol) of **3** and 0.78 g (3.12 mmol) of 4-bromobenzyl bromide (Aldrich Chemical Co.); after chromatography on a 2 × 20 cm column of silica gel, compound **4c** was obtained as a white solid; 500 mg (61%); mp 135–137 °C; FABMS (M + 1) 529; ^1H NMR (Me_2SO-d_6) δ 2.64, 2.73 (AB q, J = 14 Hz, CH_2-hydantoin), 3.73 (br s, 4 H, CH_2-phenyl), 7.25 (br d, $J_{2,3} = J_{5,6}$ = 8 Hz, 4 H, H-2 and H-6 phenyl), 7.50 (br d, $J_{2,3} = J_{5,6}$ = 8 Hz, H-3 and H-5 phenyl), 8.08 (br s, 1 H, amide NH), 10.94 (br s, 1 H, imide NH). Anal. ($C_{19}H_{18}Br_2N_2O_2S_2$) C, H, N.

5,5-Bis[[[(p-methoxyphenyl)methyl]thio]methyl]-2,4-imidazolidinedione (4d). Starting materials were 0.3 g (1.56 mmol) of **3** and 0.49 g (3.1 mmol) of 4-methoxybenzyl chloride (Aldrich); after chromatography on a 2 × 20 cm column of silica gel, compound **4d** was obtained as a white solid; 400 mg (59%); mp 122–124 °C; FABMS (M + 1) 433; ^1H NMR (Me_2SO-d_6) δ 2.63, 2.73 (AB q, J = 14 Hz, 4 H, CH_2-hydantoin), 3.71 (br s, 4 H, CH_2-phenyl), 3.73 (br s, 6 H, OCH_3), 6.87 (br d, J = 8 Hz, 4 H, H-3 and H-5 phenyl), 7.22 (br d, J = 8 Hz, 4 H, H-2 and H-6 phenyl), 8.09 (bs, 1 H, amide NH), 10.91 (br s, 1 H, imide NH). Anal. ($C_{21}H_{24}N_2O_4S_2$) C, H, N.

5,5-Bis[[[(p-cyanophenyl)methyl]thio]methyl]-2,4-imidazolidinedione (4e). Starting materials were 0.3 g (1.56 mmol) of **3** and 0.61 g (3.1 mmol) of α-bromo-*p*-tolunitrile (Aldrich); after chromatography on a 2 × 20 cm column of silica gel, compound **4e** was obtained as a white solid: 50 mg (8%); mp 164–165 °C; FABMS (M + 1) 423; ^1H NMR (Me_2SO-d_6) δ 2.66, 2.75 (AB q, J = 14 Hz, 4 H, CH_2-hydantoin), 3.94 (m, 4 H, CH_2-phenyl), 7.50 (br d, J = 8 Hz, 4 H, H-2 and H-6 phenyl), 7.80 (br d, J = 8 Hz, 4 H, H-3 and H-5 phenyl), 8.10 (br s, 1 H, amide NH), 10.97 (br s, 1 H, imide NH). Anal. ($C_{21}H_{18}N_4O_2S_2$) C, H, N.

5,5-Bis[[[(p-carboxyphenyl)methyl]thio]methyl]-2,4-imidazolidinedione (4f). Starting materials were 0.1 g (0.52 mmol) of **3** and 0.2 g (1.04 mmol) of α-bromo-*p*-toluic acid (Aldrich); after chromatography on a 2 × 20 cm column of silica gel, compound **4f** was obtained as a white solid: 90 mg (37%); mp 240–242 °C; FABMS (M + 1) 461; ^1H NMR (Me_2SO-d_6) δ 2.67, 2.77 (AB q, J = 14 Hz, 4 H, CH_2-hydantoin), 3.83 (br s, 4 H, CH_2-phenyl), 7.42 (br d, J = 8 Hz, 4 H, H-2 and H-6 phenyl), 7.89 (br d, J = 8 Hz, 4 H, H-3 and H-5 phenyl), 8.12 (br s, 1 H, amide NH), 10.95 (br s, 1 H, imide NH), 12.90 (br s, 1 H, CO_2H). Anal. ($C_{21}H_{20}N_2O_6S_2$) C, H, N.

5,5-Bis[[[[p-(methoxycarbonyl)phenyl]methyl]thio]methyl]-2,4-imidazolidinedione (4g). Starting materials were 0.3 g (1.56 mmol) of **3** and 0.71 g (3.1 mmol) of methyl 4-(bromomethyl)benzoate (Aldrich); after chromatography on a 2 × 20 column of silica gel, compound **4g** was obtained as a white solid: 300 mg (40%); mp 154–155 °C; FABMS (M + 1) 489; ^1H NMR (Me_2SO-d_6) δ 2.66, 2.76 (AB q, J = 14 Hz, 4 H, CH_2-hydantoin), 3.84 (s, 4 H, CH_2-phenyl), 3.86 (s, 6 H, CO_2CH_3), 7.44 (br d, J = 8 Hz, 4 H, H-2 and H-6 phenyl), 7.90 (br d, J = 8 Hz, 4 H, H-3 and H-5 phenyl), 8.10 (br s, 1 H, amide NH), 10.95 (br s, 1 H, imide NH). Anal. ($C_{23}H_{24}N_2O_6S_2$) C, H, N.

5,5-Bis[[[(p-nitrophenyl)methyl]thio]methyl]-2,4-imidazolidinedione (4h). Starting materials were 0.3 g (1.56 mmol) of **3** and 0.67 g (3.12 mmol) of α-bromo-*p*-nitrotoluene (Eastman Kodak); after chromatography on a 2 × 20 cm column of silica gel, compound **4h** was obtained as a white solid; 130 mg (18%); mp 154–155 °C; FABMS (M + 1) 463; ^1H NMR (Me_2SO-d_6) δ 2.67, 2.77 (AB q, J = 14 Hz, 4 H, CH_2-hydantoin),

3.89 (m, 4 H, CH_2-phenyl), 7.58 (br d, J = 8 Hz, 4 H, H-2 and H-6 phenyl), 8.11 (br s, 1 H, amide NH), 8.19 (br d, J = 8 Hz, 4 H, H-3 and H-5 phenyl), 10.96 (br s, 1 H, imide NH). Anal. ($C_{19}H_{18}N_4O_6S_2$) C, H, N.

5,5-Bis[[(4-pyridylmethyl)thio]methyl]-2,4-imidazolidinedione (4i). Starting materials were 0.1 g (0.52 mmol) of **3** and 0.17 g (1.04 mmol) of 4-picolyl chloride hydrochloride (Aldrich); after chromatography on a 2 × 20 cm column of silica gel, compound **4i** was obtained as a white solid: 110 mg (18%); mp 193–195 °C; FABMS (M + 1) 375; ^{1}H NMR (Me_2SO-d_6) δ 3.52, 3.61 (AB q, J = 14 Hz, 4 H, CH_2-hydantoin), 3.77 (s, 4 H, CH_2-pyridyl), 7.32 (dd, $J_{2,3}$ = 6 Hz, $J_{3,5}$ = 2 Hz, 4 H, H-3 and H-5 pyridyl), 8.13 (br s, 1 H, amide NH), 8.51 (dd, $J_{2,3}$ = 6 Hz, $J_{3,5}$ = 2 Hz, 4 H, H-2 and H-6 pyridyl), 10.96 (br s, 1 H, imide NH). Anal. ($C_{17}H_{18}N_4O_2S_2$·H_2O) C, H, N.

5,5-Bis[[(3-pyridylmethyl)thio]methyl]-2,4-imidazolidinedione (4j). Starting materials were 0.3 g (1.56 mmol) of **3** and 0.51 g (3.12 mmol) of 3-picolyl chloride hydrochloride (Aldrich); after chromatography on a 2 × 20 cm column of silica gel, compound **4j** was obtained as a white solid: 150 mg (26%); mp 121–122 °C dec; FABMS (M + 1) 375; ^{1}H NMR (Me_2SO-d_6) δ 2.69, 2.78 (AB q, J = 14 Hz, 4 H, CH_2-hydantoin), 3.80 (s, 4 H, CH_2-pyridyl), 7.35 (dd, $J_{4,5}$ = $J_{5,6}$ = 5 Hz, 1 H, H-5 pyridyl), 7.72 (dt, $J_{2,4}$ = 2 Hz, 1 H, H-4 pyridyl), 8.12 (br s, 1 H, amide NH), 8.45 (dd, $J_{4,6}$ = 2 Hz, 1 H, H-6 pyridyl), 8.50 (d, $J_{2,6}$ = 2 Hz, 1 H, H-2 pyridyl), 10.97 (br s, 1 H, imide NH). Anal. ($C_{17}H_{18}N_4O_2S_2$·0.3H_2O) C, H, N.

5,5-Bis[[(2-pyridylmethyl)thio]methyl]-2,4-imidazolidinedione (4k). Starting materials were 0.3 g (1.56 mmol) of **3** and 0.51 g (3.12 mmol) of 2-picolyl chloride hydrochloride (Aldrich); after chromatography on a 2 × 20 cm column of silica gel, compound **4k** was obtained as a white solid, 360 mg (62%); mp 193–195 °C dec; FABMS (M + 1) 375; ^{1}H NMR (Me_2SO-d_6) δ 2.80, 2.89 (AB q, J = 14 Hz, 4 H, CH_2-hydantoin), 3.86 (s, 4 H, CH_2-pyridyl), 7.25 (ddd, $J_{3,5}$ = 1 Hz, $J_{4,5}$ = 7 Hz $J_{5,6}$ = 6 Hz, 1 H, H-5 pyridyl), 7.48 (br d, $J_{3,4}$ = 7 Hz, $J_{3,6}$ = 1 Hz, 1 H, H-3 pyridyl), 7.75 (dd, $J_{4,6}$ = 2 Hz, 1 H, H-4 pyridyl), 8.11 (br s, 1 H, amide NH), 8.48 (m, 1 H, H-6 pyridyl), 10.90 (br s, 1 H, imide NH). Anal. ($C_{17}H_{18}N_4O_2S_2$) C, H, N.

5,5-Bis[[(1-naphthylmethyl)thio]methyl]-2,4-imidazolidinedione (4l). Starting materials were 0.3 g (1.56 mmol) of **3** and 0.55 g (3.1 mmol) of 1-(chloromethyl)naphthalene (Aldrich); after chromatography on a 2 × 20 cm column of silica gel, compound **4l** was obtained as a white solid: 220 mg (30%); mp 174 °C; FABMS (M + 1) 473; ^{1}H NMR (Me_2SO-d_6) δ 2.79, 2.88 (AB q, J = 14 Hz, 4 H, CH_2-hydantoin), 4.38 (m, 4 H, CH_2-naphthalene), 7.4–8.18 (m, 14 H, aromatics), 8.23 (br s, 1 H, amide NH), 11.20 (br s, 1 H, imide NH). Anal. ($C_{27}H_{24}N_2O_2S_2$) C, H, N.

5,5-Bis[[(2-thienylmethyl)thio]methyl]-2,4-imidazolidinedione (4m). Starting materials were 0.3 g (1.56 mmol) of **3** and 0.41 g (3.1 mmol) of 2-(chloromethyl)thiophene;[14] after chromatography on a 2.5 × 20 cm column of silica gel, compound **4m** was obtained as a white solid: 200 mg (33%); mp 170–172.5 °C; FABMS (M + 1) 385; ^{1}H NMR (Me_2SO-d_6) δ 2.72, 2.81 (AB q, J = 14 Hz, 4 H, CH_2-hydantoin), 4.01 (br s, 4 H, CH_2-thiophene), 6.95 (m, 2 H, H-3 thiophene), 6.98 (m, 2 H, H-4 thiophene), 7.43 (dd, $J_{3,5}$ = 2 Hz, $J_{4,5}$ = 5 Hz, 2 H, H-5 thiophene), 8.10 (br s, 1 H, amide NH), 10.93 (br s, 1 H, imide NH). Anal. ($C_{15}H_{16}N_2O_2S_4$) C, H, N.

5,5-Bis[[[(*p*-aminophenyl)methyl]thio]methyl]-2,4-imidazolidinedione Dihydrochloride (4n). A mixture of **4h** (0.3 g, 0.65 mmol), concentrated HCl (10 mL), and granular tin (0.15 g, 30 mesh) was heated to 100 °C until the solution became transparent. The reaction was then allowed to cool and stir at room temperature for 24 h. The solution was made basic with concentrated NH_4OH, the precipitate filtered, and the filtrate condensed in vacuo. Because the reaction salts trapped some of the product, they were combined with the condensed filtrate and chromatographed on silica gel (2.5 × 20 cm column) eluting with 95:5 $CHCl_3$–MeOH. The product-containing fractions were condensed, and the residue was converted to the dihydrochloride salt by dissolving in EtOH and adding ethanolic HCl. The solvents were decanted, and the product (extremely hygroscopic) was dried in vacuo to give 160 mg (50%) of an orange glass: mp 280 °C dec; FABMS (M + 1) 403; ^{1}H NMR (Me_2SO-d_6) δ 2.66, 2.75 (AB q, J = 14 Hz, 4 H, CH_2-hydantoin), 3.89 (br s, 4 H, CH_2-phenyl), 7.13 (br d, J = 8 Hz, 4 H, H-2 and H-6 phenyl), 7.28 (br d, J = 8 Hz, 4 H, H-3 and H-5 phenyl), 8.06 (br s, 1 H, amide NH), 10.96 (br s, 1 H, imide NH) (NH_2/NH_3^+ and H_2O in broad resonances between 3 to 4 ppm and 9 to 11 ppm). Anal. ($C_{19}H_{22}N_4O_2S_2$·2HCl·H_2O) C, H, N.

5,5-Bis[(benzoylthio)methyl]-2,4-imidazolidinedione (4o). Compound **3** (0.25 g 1.3 mmol) was dissolved in degassed pyridine (5 mL). The solution was cooled (10 °C) and benzoyl chloride (0.3 mL, 2.6 mmol) was added dropwise under a nitrogen atmosphere. The reaction mixture was stirred at room temperature overnight and then poured into ice–water. The resulting mixture was extracted twice with ethyl acetate, and the combined extracts were washed with dilute hydrochloric acid and then saturated sodium bicarbonate solution. The organic layer was dried over anhydrous sodium sulfate, azeotroped with toluene to remove pyridine, and evaporated to a gum that was chromatographed on a 2 × 20 cm column of silica gel with chloroform. The resulting white powder was triturated with ether/petroleum ether (35–60 °C) to give **4o** (0.19 g, 36%): mp 188.5–189.5 °C; FABMS (M + 1) 401; ^{1}H NMR (Me_2SO-d_6) δ 3.55, 3.64 (AB q, J = 14 Hz, 4 H, CH_2-hydantoin), 7.57 (m, 4 H, H-3 and H-5 of phenyl ring), 7.73 (m, 2 H, H-4 of phenyl ring), 7.93 (m, 4 H, H-2 and H-6 of phenyl ring), 8.27 (br s, 1 H, amide NH), 10.98 (br s, 1 H, imide NH). Anal. ($C_{19}H_{16}N_2O_4S_2$) C, H, N.

5,5-Bis[(4-pyridylthio)methyl]-2,4-imidazolidinedione (5a). Compound **5a** was prepared in a fashion analogous to **2** by reacting 1,3-dichloroacetone (1.0 g, 7.9 mmol) with 4-mercaptopyridine (1.76 g, 15.8 mmol) and then, after workup, reacting the resulting ketone with potassium cyanide (0.74 g, 11.4 mmol) and ammonium carbonate (6.45 g, 67.2 mmol) according to the literature procedure.[8] After chromatography on a 2 × 20 cm column of silica gel and recrystallization from ethanol, compound **5a** was obtained as colorless needles (320 mg, 12%): mp 235–236 °C; FABMS (M + 1) 347; ^{1}H NMR (Me_2SO-d_6) δ 3.52, 3.61 (AB q, J = 14 Hz, 4 H, CH_2-hydantoin), 7.45 (dd, $J_{2,3}$ = $J_{5,6}$ = 6 Hz, $J_{3,6}$ = $J_{2,5}$ = 2 Hz, 4 H, H-3 and H-5 pyridine), 8.12 (br s, 1 H, amide NH), 8.38 (dd, $J_{2,3}$ = $J_{5,6}$ = 6 Hz, $J_{3,6}$ = $J_{2,5}$ = 2 Hz, 4 H, H-2 and H-6 pyridine), 11.00 (br s, 1 H, imide NH). Anal. ($C_{15}H_{14}N_4O_2S_2$) C, H, N.

5,5-Bis[(phenylthio)methyl]-2,4-imidazolidinedione (5b). Compound **5b** was prepared in a fashion analogous to **2** by reacting 1,3-dichloroacetone (1.5 g, 11.8 mmol) with thiophenol (2.42 mL, 23.6 mmol) and then reacting the resulting symmetrical ketone with potassium cyanide (1.0 g, 15.4 mmol) and ammonium carbonate (4 g, 41.7 mmol) according to the literature procedure.[8] Upon cooling, a yellowish-brown solid precipitated that was filtered, washed with water and ethanol, and then crystallized from ethanol to give 3.28 g (81%): mp 155–156 °C; FABMS (M + 1) 345; ^{1}H NMR (Me_2SO-d_6) δ 3.31, 3.40 (AB q, J = 14 Hz, 4 H, CH_2-hydantoin), 7.16 (m, 10 H, aromatic), 7.98 (br s, 1 H, amide NH), 10.91 (br s, 1 H, imide NH). Anal. ($C_{17}H_{16}N_2O_2S_2$) C, H, N.

5,5-Bis[(benzylthio)methyl]-2,4-imidazolidinedithione (6). Compound **2** (0.4 g, 1.07 mmol) and 1.9 g (4.29 mmol) of finely ground phosphorus pentasulfide were refluxed 2 days in toluene (15 mL). After cooling, the reaction mixture was preadsorbed on 230–400 mesh silica gel and chromatographed on silica gel (2.0 × 20.0 cm column) eluting with chloroform. The product-containing fractions were combined and condensed in vacuo. The residue crystallized from ether/petroleum ether (35–50 °C) to give **6** as a yellow powder (80 mg, 19%): mp 130 °C; FABMS (M + 1) 405; ^{1}H NMR (Me_2SO-d_6) δ 2.87 (s, 4 H, CH_2-hydantoin), 3.76, 3.85 (AB q, J = 14 Hz, 4 H, CH_2-phenyl), 7.1–7.4 (m, 5 H, aromatic), 10.80 (br s, 1 H, amide NH), 13.44 (br s, 1 H, imide NH). Anal. ($C_{19}H_{20}N_2S_4$) C, H, N.

5-[(Benzylthio)methyl]-2,4-imidazolidinedione (7). Compound **7** was synthesized from *S*-benzyl-L-cysteine (2 g, 9.48 mmol) (Fluka) and potassium cyanate (1.7 g, 21 mmol) by a modification of the literature procedure.[16] Instead of isolating the *N*-carbamyl-*S*-benzylcysteine intermediate, after an initial reflux of 1 h, 25 mL of 10% hydrochloric acid was added and reflux was continued for 1.5 h. Upon cooling, the product precipitated as a white solid 1.7 g (77%): mp 119.5–120.5 °C; FABMS (M + 1) 237; ^{1}H NMR (Me_2SO-d_6) δ 2.73, (m, 2 H, CH_2-hydantoin), 3.78 (br s, 2 H, benzylic CH_2), 4.32 (br t, H-5 hydantoin), 7.15–7.4 (m,

5 H, aromatic), 8.01 (br s, 1 H, amide NH), 10.73 (br s, 1 H, imide NH). Anal. ($C_{11}H_{12}N_2O_2S$) C, H, N.

5-[(Benzylthio)methyl]-5-(mercaptomethyl)-2,4-imidazolidinedione (8). Compound **3** (0.60 g, 3.1 mmol) was dissolved at room temperature under N_2 in a solution made from sodium metal (143 mg, 6.2 mmol) in degassed, absolute ethanol (30 mL). Benzyl bromide (0.4 mL, 0.58 g, 3.4 mmol) was syringed dropwise into the reaction mixture, and the yellow solution was then stirred at room temperature overnight. The solvent was removed, and the crude product was preadsorbed on silica gel and chromatographed (2 × 25 cm silica gel column) with cyclohexane–ethyl acetate (1:1), followed by a second silica gel column (2 × 25 cm) using 98:2 chloroform–methanol as the eluant. After evaporation of the solvent, compound **8** was crystallized from ether/petroleum ether (35–60 °C) as a white solid (0.16 g, 18%): mp 119–120 °C; FABMS (M + 1) 283; ^{1}H NMR (Me_2SO-d_6) δ 2.35 (br t, 1 H, SH), 2.73 (m, 4 H, CH_2-hydantoin), 3.76 (br s, 2 H, CH_2-phenyl), 7.11–7.41 (m, 5 H, aromatic), 7.95 (br s, 1 H, amide NH), 10.89 (br s, 1 H, imide NH). Anal. ($C_{12}H_{14}N_2O_2S_2$) C, H, N.

5-[[(Phenylmethyl)thio]methyl]-5-[[(2-hydroxyethyl)thio]methyl]-2,4-imidazolidinedione (9). Compound **8** (0.2 g, 0.71 mmol) was dissolved at room temperature under N_2 in a solution made from sodium metal (37.6 mg, 1.6 mmol) in degassed, absolute ethanol (10 mL). 2-Bromoethanol (0.06 mL, 0.8 mmol) was syringed into the reaction mixture, which was then stirred at room temperature under a nitrogen atmosphere for 3 d. The solvent was evaporated and the crude product was preadsorbed on silica gel and chromatographed (2 × 25 cm column) on silica gel eluting with chloroform–methanol (98:2, followed by 95:5). The product-containing fractions were evaporated to give compound **9** as a white solid (0.17 g, 74%): mp 129–132 °C; FABMS (M + 1) 327; ^{1}H NMR (Me_2SO-d_6) δ 2.62 (t, J = 7 Hz, 2 H, CH_2CH$_2$OH), 2.66, 2.75 (AB q, J = 14 Hz, 2 H, CH_2SBn), 2.76, 2.85 (AB q, J = 14 Hz, 2 H, CH_2SC$_2$H$_4$OH), 3.48 (m, 2 H, CH_2OH), 3.76 (br s, 2 H, CH_2Ph), 4.80 (m, 1 H, OH), 7.20–7.39 (m, 5 H, aromatic), 8.00 (br s, 1 H, amide NH), 10.85 (br s, 1 H, imide NH). Anal. ($C_{14}H_{18}N_2O_3S_2$) C, H, N.

2,2-Bis[(benzylthio)methyl]glycine (10). Compound **10** was resynthesized by the literature method[8] by heating compound **2** (107.3 g, 0.29 mmol) and barium hydroxide monohydrate (331.7 g, 1.75 mmol) in water (2680 mL) at reflux for 192 h. The product was precipitated by acidifying the cooled solution with concentrated HCl. Filtration and drying gave 59.3 g (54%) of **10** as its dihydrate. Crystallization of a small portion from ethanol gave **10** as a white powder: mp 205–207 °C; (lit.[8] mp 205–206 °C); FABMS (M + 1) 348; ^{1}H NMR (Me_2SO-d_6) δ 2.77, 2.89 (AB q, J = 14 Hz, 4 H, CH_2-hydantoin), 3.72, 3.81 (AB q, J = 13 Hz, 4 H, CH_2Ph), 7.16–7.37 (m, 10 H, Ph), 7.51 (br s, 2 H, NH$_2$). Anal. ($C_{18}H_{21}NO_2S_2$) C, H, N.

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Registry No. 2, 32418-95-4; **3**, 142979-79-1; **4a**, 142979-78-0; **4b**, 142979-80-4; **4c**, 142979-81-5; **4d**, 142979-82-6; **4e**, 142979-83-7; **4f**, 142979-84-8; **4g**, 142979-85-9; **4h**, 142979-86-0; **4i**, 142979-87-1; **4j**, 142979-88-2; **4k**, 142979-89-3; **4l**, 142979-90-6; **4m**, 142979-91-7; **4n**, 142979-92-8; **4o**, 142979-93-9; **5a**, 142979-94-0; **5b**, 142979-95-1; **6**, 142979-96-2; **7**, 20210-01-9; **8**, 142979-97-3; **9**, 142979-98-4; **10**, 32418-96-5; PhCH$_2$OCH$_2$COCH$_2$OCH$_2$Ph, 77356-14-0; *p*-BrC$_6$H$_4$CH$_2$Br, 589-15-1; *p*-MeOC$_6$H$_4$CH$_2$Cl, 824-94-2; *p*-BrCH$_2$C$_6$H$_4$CN, 17201-43-3; *p*-BrCH$_2$C$_6$H$_4$CO$_2$H, 6232-88-8; *p*-BrCH$_2$C$_6$H$_4$CO$_2$Me, 2417-72-3; *p*-BrCH$_2$C$_6$H$_4$NO$_2$, 100-11-8; PhCOCl, 98-88-4; ClCH$_2$COCH$_2$Cl, 534-07-6; PhSH, 108-98-5; PhCH$_2$Br, 100-39-0; BrCH$_2$CH$_2$OH, 540-51-2; (bromomethyl)cyclohexane, 2550-36-9; 4-(chloromethyl)pyridine, 1822-51-1; 3-(chloromethyl)pyridine, 6959-48-4; 2-(chloromethyl)pyridine, 6959-47-3; 1-(chloromethyl)naphthalene, 86-52-2; 2-(chloromethyl)thiophene, 765-50-4; 4-pyridinethiol, 4556-23-4; *S*-benzyl-L-cysteine, 3054-01-1.