partially debrominated products. However, dehydrogenation to **2** was effected smoothly and quantitatively by treatment of **1b** with bromine in CS_2 for 3 h at room temperature. This unusual method of dehydrogenation is based on the observation that attempted monobromination of **1a** with bromine in CS_2 gave pyrene as a major product. The mechanism of this transformation apparently involves bromination on a benzylic site followed by dehydrobromination. This method of dehydrogenation has not previously been reported;[7] it may potentially be extended to other hydroaromatic compounds.

Experimental Section

2,7-Dibromo-4,5,9,10-tetrahydropyrene (1b). To a suspension of **1a** (618 mg, 3 mmol) and $FeCl_3 \cdot H_2O$ (10 mg) in water (60 mL) was added dropwise a solution of Br_2 (0.31 mL, 6 mmol) in H_2O (32 mL) over 4 h at ambient temperature. After addition was complete, the suspension was stirred overnight. During this period the solution completely decolorized. The white precipitate was filtered and dried and identified as **1b** (1.09 g, 99%) virtually pure by NMR. Crystallization from benzene gave the analytical sample of **1b** as white needles, mp 218–219 °C: NMR (500 MHz, $CDCl_3$) δ 2.82 (8, s, benzylic), 7.20 (4, s, aromatic).

Anal. Calcd for $C_{16}H_{12}Br_2$: C, 52.78; H, 3.32; Br, 43.90. Found: C, 52.65; H, 3.36; Br, 43.88.

2,7-Dibromopyrene (2). To a solution of **1b** (4.41 g, 12.1 mmol) in CS_2 (300 mL) was added dropwise Br_2 (4.26 g, 26.6 mmol) in CS_2 (300 mL) over 3 h. The reaction mixture which contained a white precipitate was stirred an additional hour. Evaporation of the solvent under reduced pressure gave virtually pure **2** (4.3 g, 99%) by HPLC on a DuPont Zorbax Sil column (4.6 mm × 15 cm) eluted with hexane (3 mL/min). Crystallization from chlorobenzene yielded 3.17 g of **2** as short white needles, mp >230 °C; NMR (500 MHz, $CDCl_3$) δ 7.98 (4, s, $H_{4,5,9,10}$), 8.28 (4, s, $H_{1,3,6,8}$).

Anal. Calcd for $C_{16}H_8Br_2$: C, 53.38; H, 2.23; Br, 44.39. Found: C, 53.63; H, 2.34; Br, 44.64.

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Registry No. **1a**, 781-17-9; **1b**, 17533-36-7; **2**, 102587-98-4.

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N^4-(Malonyl-D-cysteinyl)-L-2,4-diaminobutyrate: The End-Group-Modified Retro-Inverso Isomer of Glutathione

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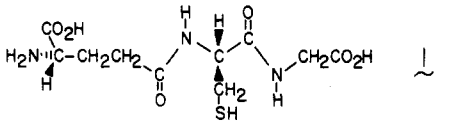
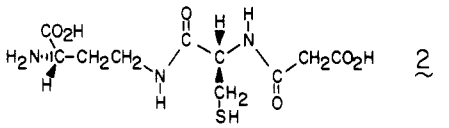
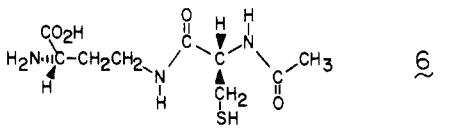
The tripeptide glutathione, **1** (GSH), is an important and ubiquitous cofactor in biological systems. It is a substrate or product for more than a dozen enzymes in conjuction with its role as a redox buffer and its involvement in amino acid transport and in the detoxication of electrophiles.[1] Most enzymes which catalyze chemistry at the sulfhydryl group of the peptide are very specific for GSH. For instance, the only known alternative substrate for GSH with the glutathione *S*-transferase is homoglutathione.[2] In this paper we report the preparation of the end-group-modified retro-inverso isomer[3] of GSH, **2** (rGSH), and a related *retro*-peptide, **6**, and the observation that **2** and **6** are substrates for several enzymes that participate in the biochemistry of the sulfhydryl group of GSH.







Synthesis of **2** is straightforward commencing with the ethyl chloroformate mediated condensation of N^2-tosyl-L-2,4-diaminobutyrate benzyl ester with *N*-*t*-BOC-*S*-benzyl-D-cysteine to give the protected dipeptide **3**. Removal of the *t*-BOC group with anhydrous CF_3CO_2H and coupling of the resulting dipeptide **4** with monobenzyl malonate in the presence of dicyclohexylcarbodiimide gave the fully protected tripeptide **5** in 66% overall isolated yield from D-cysteine. Complete deprotection of **5** in CF_3CO_2H with 1 M CF_3SO_3H and 2.6 M thioanisole gave a 65% isolated yield of **2** with <10% of the decarboxylated product **6**, after aqueous workup and purification by preparative HPLC. Decarboxylated peptide **6** was isolated in 40–50% yield as the exclusive sulfhydryl-containing peptide upon removal of CF_3CO_2H and thioanisole under vacuum, before workup. Oxidation of **2** with diamide gave the disulfide rGSSGr, **7**, in quantitative yield.

Structures of peptides **2**, **6**, and **7** were confirmed by ^1H and ^{13}C NMR and circular dichroism spectroscopy. Of particular interest was the exchange behavior of the malonyl methylene protons of **2** and **7**. The carboxy-terminal methylene protons of **2** [δ 3.42 (s, 2 H)] exchanged in D_2O (pD ~1.7, 23 °C) with a $t_{1/2}$ of 1–2 h with concomitant collapse of the singlet ^{13}C resonance at 42.6 ppm in the ^1H-decoupled spectrum to a 1:2:2:1 quartet at 42.3 ppm ($^1J_{^2H,^{13}C}$ = 21 Hz). Similar behavior is observed for the two malonyl residues of **7**, where the two pairs of diastereotopic methylene protons [H_A, δ 3.09 (d, 2 H, ^2J = 15.9 Hz); H_B, δ 3.23 (d, 2 H, ^2J = 15.5 Hz)] exchange with significantly different rate constants, $k^{H_A} = 3.4 \times 10^{-4}$ s^{-1} and $k^{H_B} = 7.7 \times 10^{-5}$ s^{-1}, at pH 4.1 and 21 °C. Both **2** and **7** are unstable at low pH. Prolonged storage of either at pH <2 results in substantial decarboxylation.

Circular dichroic transitions of the peptide backbones (190–200 nm) of the *retro*-peptides **2** and **7** (Figure 1) are of opposite sign to those of **1** and its disulfide **8** as might be expected for molecules with mirror-image peptide backbones. The reason for the large difference in absolute magnitude of the transitions at 200 nm of **1** and **2** is not clear but may be due to contributions from the distal chiral center of the two molecules or population of different conformational states.

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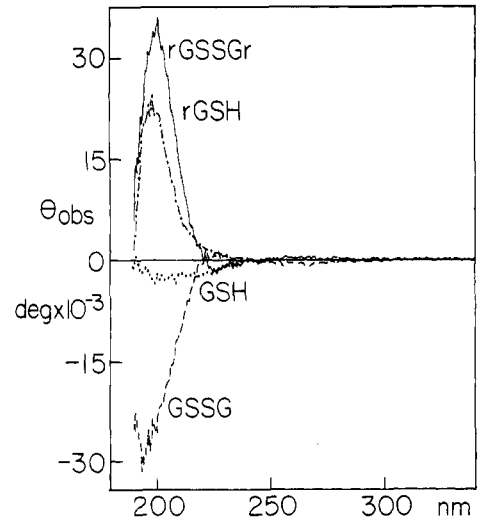

Figure 1. Circular dichroism spectra of 33 μM each 1 (···), 2 (—·—), 7 (—), and 8 - - -) in H_2O.

Table I. Comparison of Kinetic Parameters for Enzyme-Catalyzed Reactions of Glutathione and *retro*-Glutathione Analogues

enzyme	peptide	k_c, s^{-1}	k_c/K_m, M^{-1} s^{-1}
glutathione	1	2.6 ± 0.1	(4.3 ± 0.2) × 10^4
S-transferase 3-3	2	0.036 ± 0.004	47 ± 8
	6	0.013 ± 0.002	4.0 ± 0.4
glutathione	1	0.63 ± 0.02	(1.1 ± 0.1) × 10^4
S-transferase 4-4	2	0.027 ± 0.007	3.5 ± 0.4
	6	0.019 ± 0.002	9.0 ± 0.7
glyoxalase I	1	400 ± 10	(6.5 ± 0.3) × 10^5
	2	3.5 ± 0.8	290 ± 30
	6	*a*	120 ± 4
glutathione	1		(1.7 ± 0.1) × 10^5
peroxidase[b]	2		(4.8 ± 0.4) × 10^3
	6		(5.0 ± 0.5) × 10^3
glutathione	8	130 ± 10	(1.2 ± 0.1) × 10^6
reductase	7	*a*	5.3 ± 1.5

[a] Accessible substrate concentrations were $\ll K_m$ such that k_c was not well determined. [b] Second-order constants were obtained from the quotient of the observed first-order rate constant and the active site concentration.

retro-Peptides 2 and 7, which are topochemically similar to 1 and 8, represent a new class of glutathione analogues that are apparently unique in that they serve as alternate substrates for several glutathione-requiring enzymes which catalyze reactions involving the sulfhydryl group. As illustrated in Table I, 2 is a substrate for glutathione *S*-transferase, glutathione peroxidase and glyoxalase I with k_c and k_c/K_m values of 10^2- to 10^3-fold lower than the natural peptide. Interestingly, the decarboxylated peptide is also a substrate mimic of 1 with these three enzymes. The disulfide 7 is an alternative substrate, albeit a very poor one for glutathione reductase. The kinetic results demonstrate that, to a first approximation, the correct orientation of the peptide bonds of 1 and 8 are important though not essential for substrate recognition and catalysis with several glutathione requiring enzymes.

These observations suggest that *retro*-peptide analogues of glutathione may be of considerable and general importance in studies of the chemical and kinetic mechanisms of glutathione-requiring enzymes. In addition, the fact that 2 and its S-substituted derivatives should be refractory toward enzymes which process the peptide bonds of 1 (e.g., γ-glutamyl transpeptidase) suggests that such compounds might be interesting in biological studies. For instance, *retro*-glutathionyl analogues of the leukotrienes are of potential value in elucidating the biochemistry of anaphylaxis.

Experimental Section

N^2-Tosyl-N^4-(N-*t*-BOC-*S*-benzyl-D-cysteinyl)-L-2,4-diaminobutyrate Benzyl Ester (3). To a stirred solution of 6.22 g (20 mmol) of N-*t*-BOC-*S*-benzyl-D-cysteine[4] (Chemical Dynamics Corp.) and 2.79 mL of $(CH_3CH_2)_3N$ in 40 mL of CH_2Cl_2 at −10 °C under N_2 was added 1.92 mL of $ClCO_2CH_2CH_3$. After 15 min a precooled solution of 8.0 g of N^2-tosyl-L-2,4-diaminobutyric acid benzyl ester hydrochloride[5] and 2.79 mL of $(CH_3CH_2)_3N$ in 150 mL of CH_2Cl_2 were added slowly via a cannula. The mixture was stirred at −5 °C for 20 min and then at room temperature for 2 h. Dilution with 250 mL of CH_2Cl_2 followed by washing with 5% HCl, 5% $NaHCO_3$, and water, drying over Na_2SO_4, and evaporation of CH_2Cl_2 gave a 11.125 g (17.0 mmol) of 3: mp 158–161 °C; $[\alpha]^{23}D$ +13.8° ($CHCl_3$, *c* 0.05). Anal. Calcd for $C_{33}H_{42}S_2N_3O_7$ (656.90): C, 60.33; H, 6.44; N, 6.40; S, 9.77. Found: C, 60.38; H, 6.38; N, 6.47; S, 9.84. ^1H NMR (400 MHz, $CDCl_3$, reference external Me_4Si) δ 1.42 (s, 9 H), 1.57 (m, 1 H), 2.15 (m, 1 H), 2.39 (s, 3 H), 2.71 (dd, 1 H, ^2J = 13.8 Hz, ^3J = 6.2 Hz), 2.89 (dd, 1 H, ^2J = 13.8 Hz, ^3J = 5.6 Hz), 3.27 (m, 1 H), 3.70 (m, 1 H), 3.63 (d, 1 H, ^2J = 13.4 Hz), 3.69 (d, 1 H, ^2J = 13.4 Hz), 4.02 (m, 1 H, ^3J = 3.8 Hz, ^3J = 11.0 Hz, $^3J_{NH-CH}$ = 9.3 Hz), 4.24 (br m, 1 H, ^3J = 5.6 Hz, ^3J = 6.2 Hz, $^3J_{NH-CH}$ = 7.1 Hz.), 4.84 (dd, 1 H, ^2J = 15.9 Hz), 4.88 (d, 1 H, ^2J = 15.9 Hz), 5.27 (br d, 1 H, *J* = 7.2 Hz), 5.32 (d, 1 H, *J* = 9.3 Hz), 6.91 (br dd, 1 H, *J* = 7.0 Hz, *J* = 6.9 Hz), 7.06–7.74 (m, 14 H).

N^2-Tosyl-N^4-(N-benzylmalonyl-*S*-benzyl-D-cysteinyl)-L-2,4-diaminobutyric Acid Benzyl Ester (5). To a suspension of 3 (9.83 g, 15 mmol) in 30 mL of glacial acetic acid at 0 °C was added 70 mL of anhydrous CF_3CO_2H. The mixture was stirred for 30 min at room temperature, and the solvents were removed by flash evaporation. The product was tritrated and washed three times with ether and dried in vacuo to yield 8.3 g (83%) of the deblocked dipeptide 4 as its trifluoroacetate salt. Crude 4, which was very hygroscopic, was used without further purification.

To a solution of 8.0 g (12 mmol) of 4, 2.33 g (12 mmol) of monobenzyl malonate, and 1.68 mL of $(CH_3CH_2)_3N$ in 100 mL of CH_2Cl_2 was added 2.50 g dicyclohexylcarbodiimide. The mixture was stirred under N_2 at room temperature overnite (~10 h). After addition of 0.3 mL of CH_3CO_2H, dicyclohexylurea was removed by filtration and washed with 50 mL of CH_2Cl_2. The combined filtrates were washed with two 50-mL portions of 5% HCl, 5% $NaHCO_3$, and H_2O and finally dried over anhydrous sodium sulfate. Evaporation of the solvent gave an off-white powder, which was dissolved in ethyl acetate and crystallized with hexane to give 8.25 g (11.3 mmol) of the fully protected tripeptide 5: $[\alpha]^{23}D$ +23.2° ($CHCl_3$, *c* 0.053). Anal. Calcd for $C_{38}H_{41}S_2N_3O_8$ (731.61): C, 62.38; H, 5.60; N, 5.74; S, 8.77. Found: C, 62.56; H, 6.04; N, 6.10; S, 8.68. ^1H NMR (400 MHz, $CDCl_3$, reference external Me_4Si) δ 1.57 (m, 1 H), 2.18 (m, 1 H), 2.38 (s, 3 H), 2.73 (dd, 1 H, ^2J = 13.8 Hz, ^3J = 5.4 Hz), 3.08 (dd, 1 H, ^2J = 13.8 Hz, ^3J = 5.0 Hz), 3.27 (m, 1 H), 3.36 (d, 1 H, ^2J = 17.5 Hz), 3.45 (d, 1 H, ^2J = 17.5 Hz), 3.62 (d, 1 H, ^2J = 13.5 Hz), 3.70 (d, 1 H, ^2J = 13.5 Hz), 3.67 (m, 1 H), 4.05 (m, 1 H, ^3J = 3.9 Hz, ^3J = 11.0 Hz, $^3J_{NH-CH}$ = 9.2 Hz), 4.65 (m, 1 H, ^3J = 5.4 Hz, ^3J = 5.0 Hz, $^3J_{NH-CH}$ = 7.8 Hz), 4.84 (d, 1 H, ^2J = 12.1 Hz), 4.79 (d, 1 H, ^2J = 12.1 Hz), 5.12 (d, 1 H, ^2J = 12.2 Hz), 5.17 (d, 1 H, ^2J = 12.2 Hz), 5.34 (d, 1 H, *J* = 9.6 Hz), 7.13 (dd, *J* = 6.6 Hz, *J* = 3.8 Hz), 7.65 (d, 1 H, *J* = 7.8 Hz), 7.06–7.72 (m, 19 H).

N^4-(Malonyl-D-cysteinyl)-L-2,4-diaminobutyrate (2). The protected peptide (250 mg, 0.34 mmol) under Ar in a 25-mL round-bottom flask was dissolved with stirring at 0 °C in 5.0 mL of anhydrous CF_3CO_2H. Thioanisole (2.4 mL, 20.9 mmol) was added followed by 0.7 mL (7.91 mmol) CF_3SO_3H.[6] This mixture, originally brownish red in color turned yellow-green after ~30

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min at 0 °C. The mixture was allowed to warm to room temperature and stirred for 6 h, after which it developed a deep red color. The bulk of the CF_3CO_2H was removed by flash evaporation at 25 °C and the residue rapidly poured into a chilled (0 °C) flask containing a rapidly stirred mixture of 25 mL of CH_2Cl_2 and 75 mL of ice and water through which N_2 was vigorously bubbled. The aqueous layer was quickly adjusted pH 7.5 with dilute NH_4OH, transfered to a separatory funnel, and extracted four times with 50-mL portions of CH_2Cl_2 under N_2. N_2 was bubbled through the aqueous layer for ~15 min until the solution was clear. The aqueous layer was shell frozen and lyophilized. The product was purified by preparative HPLC (2.12 × 25 cm Dupont Zorbax ODS, 2.0 mL/min, 0.01 M CF_3CO_2H), $k' = 1.53$. Fractions (5 mL) were collected and monitored at 412 nm after reaction of a 50-μL aliquot with 5,5'-dithiobis(2-nitrobenzoic acid) at pH 8.0. The only sulfhydryl containing material other than **2** detected was **6** ($k' = 1.76$), which was generally present to the extent of 10% or less. Fractions containing **2** were pooled and lyophilized to give 0.23 mmol (66%) of **2** as the trifluoroacetate salt. The final product was pure by the criteria of HPLC under the above conditions and by quantitative SH group analysis: ^1H NMR (400 MHz, D_2O, pD ~1.7, reference external DSS) δ 1.98–2.16 (m, 2 H), 2,4-diaminobutyryl-β-CH_2), 2.85 (d, 2 H, J = 6.2 Hz, cys-β-CH_2), 3.29–3.45 (m, 2 H, 2,4-diaminobutyryl-γ-CH_2), 3.42 (s, 2 H), malonyl-CH_2), 3.94 (t, 1 H, J = 6.6 Hz, 2,4-diaminobutyryl-α-CH), 4.41 (t, 1 H, J = 6.2 Hz, cys-α-CH) [after 16 h in D_2O resonance at δ 3.42 is absent]; ^{13}C NMR (100 MHz, H_2O, pH ~2, reference external dioxane) 26.0 (cys-C_β), 30.3 (diaminobutyryl-C_β), 36.3 (diaminobutyryl-C_γ), 42.6 (malonyl-CH_2), 51.6 (diaminobutyryl-C_α), 56.8 (cys-Cα), 168.9, 172.4, 172.7, 173.1 ppm (carbonyl carbons) [after 16 h in D_2O malonyl-CD_2 resonance appears at 42.3 ppm as a 1:2:2:1 quartet (J = 21 Hz)].

***N*⁴-(*N*-Acetyl-D-cysteinyl)-L-2,4-diaminobutyrate (6).** The decarboxylated peptide **6** was prepared from **5** simply by altering the workup in the synthesis of **2** above. Thus 250 mg of **5** was deprotected as above under Ar for 8 h at ambient temperature. CF_3CO_2H was removed by flash evaporation at 40 °C, and the residue was placed in a vacuum desiccator overnite. Aqueous workup under N_2 at room temperature followed by lyophilization and purification by HPLC gave **6** as the trifluoroacetate salt (0.16 mmol) as the only sulfhydryl containing product, which was pure by HPLC ($k' = 1.76$) and quantitative SH group titration: ^1H NMR (200 MHz, D_2O, pD ~2.0, reference external DSS) δ 2.16 (s, 3 H, acetyl-CH_3), 2.18–3.34 (m, 2 H, 2,4-diaminobutyryl-β-CH_2), 3.00 (d, 2 H, J = 6.3 Hz, cys-β-CH_2), 3.49–3.59 (m, 2 H, 2,4-diaminobutyryl-γ-CH_2), 4.16 (t, 1 H, J = 6.7 Hz, 2,4-diaminobutyryl-α-CH), 4.52 (t, 1 H, J = 6.3 Hz); ^{13}C NMR (100 MHz, D_2O, pD ~2.0, reference external dioxane) 22.6 (acetyl-CH_3), 25.9 (cys-C_β), 30.2 (diaminobutyryl-C_β), 36.2 (diaminobutyryl-C_γ), 51.4 (diaminobutyryl-C_α), 56.7 (cys-C_α), 175.3, 173.4, 172.2 ppm (carbonyl carbons).

***retro*-Glutathione Disulfide (7).** A stirred solution of 24 mg of **2** (78 μmol) in 1.2 mL of H_2O was adjusted to pH 7.2 with 1 N NaOH under N_2 and 7 mg (41 μmol) of diamide[7] was added. After 2 h at room temperature the disulfide was isolated by preparative HPLC (1.0 × 25 cm Altex Ultrasphere ODS, 2 mL/min, 0.1 M ammonium acetate, pH 4.1) monitored at 240 nm. Repeated lyophilization of the peak ($k' = 1.66$) containing the peptide gave 23 mg (37.6 μmol) of **7**, 96% from **2**: ^1H NMR (400 MHz, D_2O, pD ~3.0, reference external DSS) δ 1.90 (m, 2 H, 2,4-diaminobutyryl-β-CH_2, H_A), 2.00 (m, 2 H, 2,4-diaminobutyryl-β-CH_2, H_B), 2.91 (dd, 2 H, ^2J = 14.2 Hz, ^3J = 8.7 Hz, cys-β-CH_2, H_A), 3.16 (dd, 2 H, ^2J = 14.4 Hz, ^3J = 4.8 Hz, cys-β-CH_2, H_B), 3.09 (d, 2 H, ^2J = 15.9 Hz, malonyl-CH_2, H_A), 3.23 (d, 2 H, ^2J = 15.5 Hz, malonyl-CH_2, H_B), 3.20–3.33 (m, 4 H, 2,4-diaminobutyryl-γ-CH_2), 3.58 (t, 2 H, J = 7.1 Hz, 2,4-diaminobutyrl-α-CH), 4.62 (dd, 2 H, ^3J = 4.8 Hz, ^3J = 8.2 Hz, cys-α-CH) [after 24 h in D_2O doublets at δ 3.09 and 3.23 are absent]; ^{13}C NMR (100 MHz, H_2O, pH ~3.0, reference external dioxane) 30.9 (diaminobutyryl-C_β), 36.8 (diaminobutyryl-C_γ), 39.3 (cys-C_β), 45.9 (malonyl-CH_2), 53.4 (diaminobutyryl-C_α), 53.8 (cys-C_α), 172.6, 173.3, 174.8, 175.7 ppm (carbonyl carbons) [after 24 h in D_2O resonance at 45.9 ppm is absent].

Enzyme-Catalyzed Reactions. Rat liver glutathione *S*-transferases were purified as previously described.[8] Yeast glyoxalase I, Se-containing bovine erythrocyte glutathione peroxidase, and spinach glutathione reductase were from Sigma Chemical Co. Glutathione *S*-transferase was assayed as previously described at pH 7.3 with 50 and 200 μM 1-chloro-2,4-dinitrobenzene for isozyme 3-3 and 4-4, respectively.[9] Glyoxalase I reactions were followed at 240 nm using 20 mM methylglyoxal in 0.15 mM sodium phosphate (pH 6.5). All thiohemiacetal concentrations were computed by using K_D = 3 mM previously determined for glutathionyl hemiacetal formation.[10] Glutathione peroxidase catalyzed reactions were performed in 0.1 M sodium phosphate and 1 mM EDTA (pH 7.0) with 0.54 mM *tert*-butyl hydroperoxide.[11] Reactions were monitored by following the loss of reduced thiol by SH group titration. The kinetics were strictly first order in **1**, **2**, or **6**.[12] Glutathione reductase reactions were monitored at 340 nm by using 150 μM NADPH at pH 6.5. Initial velocities were determined at 25 °C, and kinetic parameters were calculated using the program HYPER.[13] Initial velocities were corrected for spontaneous reactions when necessary. In all cases initial velocities of the enzyme-catalyzed reactions were at least ten times spontaneous reaction rates. That reactions were not due to contamination of enzymes with adventitious **8** which was reduced to **1** upon addition of **2** or **6** was ruled out by complete consumption of **2** and **6** in reactions run to completion and isolation of products.

Instrumental Methods. ^1H nuclear magnetic resonance spectra were obtained at 400 or 200 MHz on a Bruker AM-400 or IBM WP 200 SY spectrometer, respectively. Coupling constants assignments were aided by homonuclear decoupling experiments. ^{13}C spectra were obtained at 100 MHz with broad-band proton decoupling. Circular dichroism spectra were obtained on a JASCO J-500C spectropolarimeter. Sample concentrations were determined by SH group titration or UV absorption at 230 nm. Chemical purity was determined by HPLC.

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