mg, 27%), and 4 (17 mg, 46%). **33**: ^{1}H NMR ($CDCl_3$) δ 0.02 (s, 9 H), 1.27 (d, 2 H, J = 9 Hz), 1.60 (s, 3 H), 3.27 (s, 3 H), 3.30 (s, 3 H), 3.10–3.22 (m, 1 H), 4.74–4.90 (m, 2 H), 6.80 (s, 1 H); IR (neat) 1700, 1660, 1450 cm^{-1}; exact mass calcd for $C_{14}H_{24}N_2O_2Si$ 280.1606, found 280.1599. **34**: ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 6 H), 3.30 (s, 3 H), 3.36 (s, 3 H), 5.00 (d, 1 H, J = 17 Hz), 5.03 (d, 1 H, J = 9 Hz), 6.04 (dd, 1 H, J = 9, 17 Hz), 6.92 (s, 1 H); IR (neat) 1700, 1650, 1350 cm^{-1}; exact mass calcd for $C_{11}H_{16}N_2O_2$ 208.1211, found 208.1201.

Acknowledgment. This work was supported by a Grant-in-Aid for Specially Promoted Research from the Ministry of Education of Japan (No. 61065003). We are grateful to Yamasa Shoyu Co., Ltd., for a generous gift of uridine derivatives.

Registry No. **1**, 40738-83-8; **2**, 762-72-1; **3**, 105183-69-5; **4**, 874-14-6; **4** (5-fluoro), 3013-92-1; **5**, 1024-99-3; **6**, 54-42-2; **7**, 59240-49-2; **8**, 73-39-2; **9**, 58-96-8; **10**, 951-78-0; **11**, 21428-19-3; **11** (R = I), 21418-68-8; **12**, 105183-68-4; **13**, 99044-61-8; **14**, 105183-70-8; **15**, 18293-99-7; **16**, 99044-62-9; **17**, 105183-76-4; **18**, 1192-37-6; **19**, 105183-71-9; **20**, 105183-73-1; **21**, 625-27-4; **22**, 105183-72-0; **23**, 105183-74-2; **29**, 105183-75-3; **33**, 99044-64-1; **34**, 99044-63-0; $PhCH_2SiMe_3$, 770-09-2; $(Me(CH_2)_3)_3SnCH_2CH{=}CH_2$, 24850-33-7.

Preparation of β,β-Dialkyl Analogues of Cysteine Suitable for Peptide Synthesis

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Received April 7, 1986

A general method is described for the preparation of cysteine derivatives that are substituted with one or two alkyl groups at the β-carbon. The synthesis is based on the sulfenylation of N^{α}-formyl-α,β-dehydro amino acid esters. The protected dehydro esters were synthesized by the condensation of ethyl isocyanoacetate with a ketone. The sulfenylation of these compounds was accomplished by refluxing with phosphorus pentasulfide to form the intermediate thiazoline, which can be hydrolyzed to the hydrochloride salt of the free sulfhydryl amino acid by heating in acid. The free sulfhydryl amino acid salt was protected as the *S*-*p*-methylbenzyl thioether, isolated as the zwitterion. The S-protected amino acids were then protected as the N^{α}-*tert*-butyloxycarbonyl derivatives and are suitable for use in solution- or solid-phase peptide synthesis.

Peptides comprise a large class of biologically active molecules, which, until recently, have been difficult to prepare synthetically. Within the past several years, advances in the chemistry of both solid- and solution-phase peptide synthesis have greatly facilitated the preparation of specific peptide analogues.[1] Additionally, rational design of peptide hormones to possess specific biological properties (i.e., increased potency, prolonged activity, antagonism, and receptor specificity) is now feasible.[2,3] Since peptide hormones are generally highly flexible molecules, with a myriad of possible conformations—of which only one or a limited number may be responsible for the observed biological response—it is often necessary to reduce the number of possible conformations in order to induce a specific biological response or property.

For the past several years, we have attempted to design "biologically specific" peptide hormones through conformational restriction of the mobility of the molecule (e.g., side-chain or backbone restriction). Specific examples of conformational restriction include the substitution of nonproteinogenic rigid or semirigid amino acids into a peptide hormone[4] and cyclization of a peptide, either between individual side chains[5] or between the N- and the C-terminus.[6] In general, for an amino acid substitution to impart significant conformational constraint on a peptide, the amino acid itself must be conformationally restricted. This approach has been expanded upon and employed in our laboratory for the preparation of conformationally restricted disulfide-linked peptides by the substitution of β,β-dimethylcysteine (penicillamine) for cysteine in enkephalin,[7] somatostatin,[8] and oxytocin.[9] Specifically in enkephalin, this substitution led to the preparation of a receptor-specific peptide hormone, [D-Pen^2,D-Pen^5]enkephalin (DPDPE), the most δ-selective enkephalin agonist known.[7] In this molecule, conformational restriction is induced by disulfide bond formation between the penicillamine (β,β-dimethylcysteine) residues in positions 2 and 5 and by the geminal dimethyl groups. This method of conformational restriction has also been used in fragments of somatostatin to prepare analogues that exhibit a high degree of antagonistic activity at the μ opioid receptor.[8] As part of an investigation aimed at preparing more highly constrained sulfur amino acids for incorporation into peptide hormones, we report a general and relatively simple synthesis of nonproteinogenic β,β-disubstituted sulfur amino acids. These amino acids have been subsequently orthogonally protected at the α amine and the sulfur functionalities, such that they are suitable

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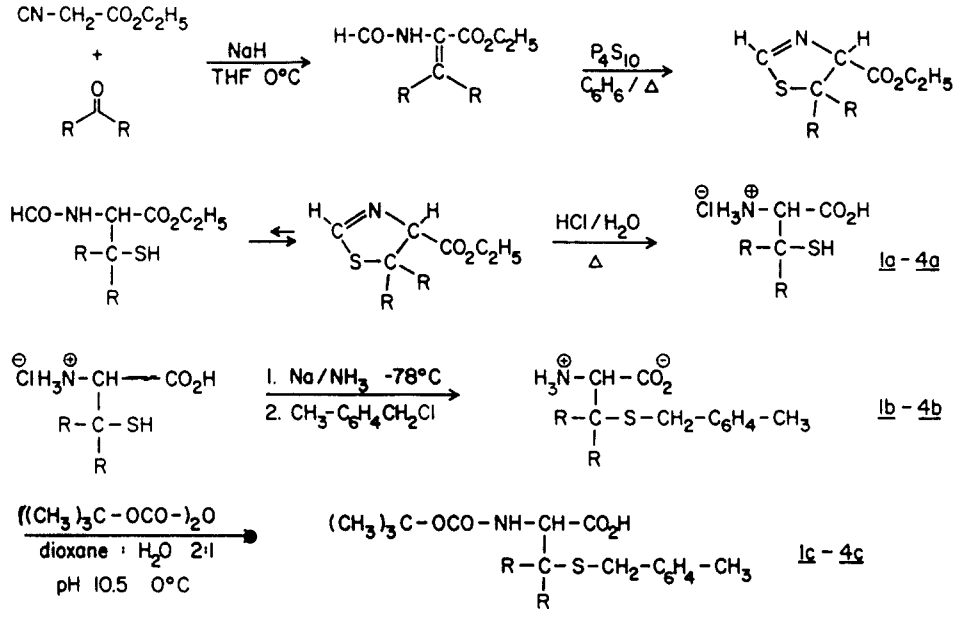
Scheme I



for solid- or solution-phase peptide synthesis.

Previous efforts to prepare these amino acids, including (1) Michael addition of sulfur nucleophiles to suitably protected dehydro amino acids[10] and (2) condensation of glycine equivalents with thioketones,[11] met with only limited success. We report here the reaction of phosphorus pentasulfide with N^{α}-formyl-protected dehydro amino acid esters (prepared by the methodology of Schöllkopf et al.[12,13]) to prepare N^{α}-formyl-β,β-substituted cysteine analogues (Scheme I). Following acid-catalyzed hydrolysis, the unprotected sulfur amino acids were suitably protected for either solution- or solid-phase peptide synthesis.

Results and Discussion

The fully protected (suitable for peptide synthesis) β,β-dialkylcysteine derivatives could be prepared in overall yields of 10% to 30% by starting from ethyl isocyanoacetate and utilizing the synthetic scheme outlined in Scheme I. The initial condensation of ethyl isocyanoacetate with a given ketone proceeded in only moderate (<50%) yield and in many cases proved to be the limiting step in the reaction scheme (See Experimental Section).

Schöllkopf and Hoppe reported[10] that 2-thiazoline-4-carboxylate esters were formed "in a less pure state" (as compared with a second method) by refluxing a benzene solution of an N^{α}-formyl-α,β-dehydro amino acid ester with phosphorus pentasulfide (P_4S_{10}) for 5 h. In our hands, the reaction of P_4S_{10} with the N^{α}-formyl-α,β-dehydro amino acid ester proceeded rapidly (1–2 h) in gently refluxing benzene; starting material was completely converted to a mixture of 2-thiazoline-4-carboxylate and N^{α}-formyl-β-sulfhydryl amino acid ester (Scheme I). The free sulfhydryl form is a minor component; however, its presence is verified by TLC, NMR, and the Ellman test.[14] Attempts to separate the free thiol and thiazoline were unsuccessful and apparently led to the decomposition of the thiazoline. However, separation was not necessary, since following evaporation of the benzene at reduced pressure the mixture of crude thiazoline and free thiol can be hydrolyzed directly in gently refluxing aqueous hydrochloric acid (4 N). The protected amino acid esters were completely hydrolyzed to the free sulfhydryl, free carboxylate, and amine hydrochloride overnight, with the exception of the (3-*tert*-butylpentamethylene)cysteine, which required 4 days for complete hydrolysis of the ethyl ester (see Experimental Section). This is probably a result of the high lipophilicity of this analogue, which renders the protected or unprotected forms virtually insoluble in dilute aqueous hydrochloric acid.

The sulfhydryl amino acid hydrochlorides were protected such that they were suitable for solid-phase or solution peptide synthesis. The free thiol was initially protected as the *S*-*p*-methylbenzyl thioether by reaction with α-chloro-*p*-xylene in sodium and liquid ammonia (Scheme I).[15] Precipitation of the amino acid from water yielded a pure protected product in good yield (>70%). (Due to the physical properties of the 3-*tert*-butylpentamethylene analogues, a slightly different procedure was required (see Experimental Section).)

Finally, in preparation for peptide synthesis, the α amino group was protected as its *tert*-butoxycarbonyl derivative (*t*-Boc). This derivatization was performed relatively simply by reaction with di-*tert*-butyl dicarbonate in aqueous dioxane.[16,17] The incorporation of these amino acids into cyclic peptides is in progress and will be reported later.

In general, this synthetic methodology can be utilized to obtain reasonable yields of β,β-alkyl-substituted analogues of cysteine relatively easily. In this paper, we report that the following sulfur amino acids have been prepared and fully protected, suitable for incorporation into peptides via solid-phase or solution peptide synthesis: (1) β,β-diethylcysteine, (2) β,β-tetramethylenecysteine, (3) β,β-pentamethylenecysteine, and (4) β,β-(3-*tert*-butylpentamethylene)cysteine (Figure 1).

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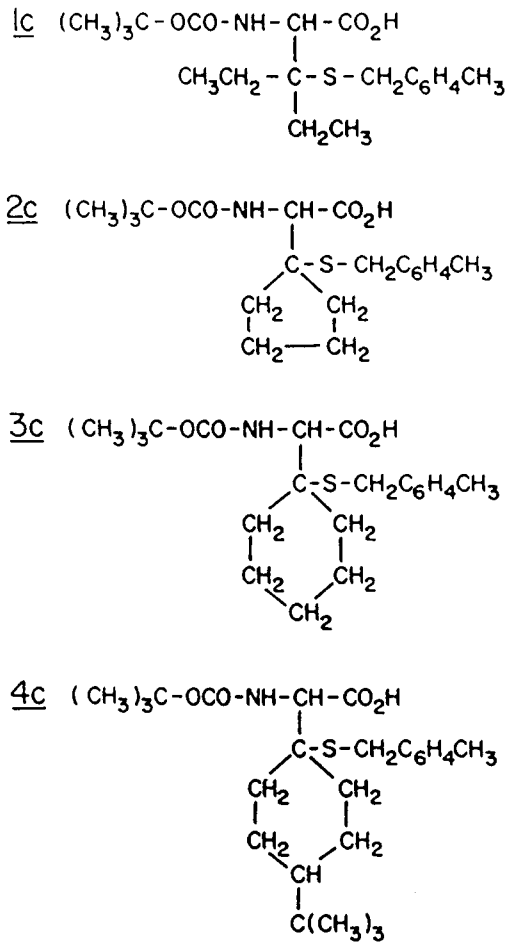

Figure 1. The structures of N^{α}-*t*-Boc-*S*-(*p*-methylbenzyl)-β,β-diethylcysteine (**1c**), N^{α}-*t*-Boc-*S*-(*p*-methylbenzyl)-β,β-tetramethylenecysteine (**2c**), N^{α}-*t*-Boc-*S*-(*p*-methylbenzyl)-β,β-pentamethylenecysteine (**3c**), and N^{α}-*t*-Boc-*S*-(*p*-methylbenzyl)-β,β-(3-*tert*-butylpentamethylene)cysteine (**4c**).

Experimental Section

Tetrahydrofuran was distilled from sodium benzophenone ketyl before use and stored under argon. Sodium hydride, phosphorus pentasulfide, 3-pentanone, 4-*tert*-butylcyclohexanone, and ethyl isocyanoacetate were obtained from Aldrich and were used as received. Cyclopentanone and cyclohexanone (Aldrich) were distilled before use and stored over molecular sieves. Reactions involving air-sensitive compounds were done under positive argon pressure.

All 60-MHz proton NMR were obtained on a Varian T-60; high-field (250-MHz) proton NMR were obtained on a Bruker WM-250 spectrometer. Melting points were determined in open capillary tubes on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were performed by University Analytical or by Micanal, Tucson, AZ. All yields refer to isolated yields of pure products. Ninhydrin[18] and Ellman[14] tests were performed in order to test for free amine and free sulfhydryl groups, respectively.

The same general procedure described was used for the synthesis of the *S*-*p*-methylbenzyl-protected derivatives of β,β-diethylcysteine (**1b**), β,β-tetramethylenecysteine (**2b**), and β,β-pentamethylenecysteine (**3b**). The details of the synthesis of *S*-(*p*-methylbenzyl)-β,β-(3-*tert*-butylpentamethylene)cysteine (**4b**) are described separately because the synthesis of this compound required significant modification of the general procedure.

General Procedure for the Synthesis of the N^{α}-Formyl-α,β-dehydro Amino Acid Esters in Scheme I. The synthetic methods utilized to prepare the precursor dehydro amino acid esters to **1a**, **2a**, **3a**, and **4a** were similar to those previously reported in the literature.[12,13] The following general scheme was used. To a dry, three-neck, 100-mL round-bottom flask equipped with stir bar and two addition funnels was added sodium hydride (1.0 g of a 60% dispersion, 25 mmol) and dry THF (25 mL). The first addition funnel was charged with ethyl isocyanoacetate (2.5 g, 2.4 mL, 22 mmol) in 10 mL of THF, and the second funnel contained the appropriate ketone (3-pentanone, cyclopentanone, cyclohexanone, or 4-*tert*-butylcyclohexanone, respectively) in 10 mL of THF. The sodium hydride/THF mixture was cooled to 0 °C under argon, and the ethyl isocyanoacetate and ketone solutions were added dropwise simultaneously at approximately identical rates over a 20–30-min period. The temperature was kept at 0 °C throughout. After the additions were completed, the reaction mixture was allowed to warm from 0 °C to room temperature with stirring. The mixture was then stirred for 2–4 h at 27 °C. Brine or saturated ammonium chloride (5–10 mL) was added cautiously and the quenched reaction mixture separated, and the aqueous layer was extracted with 2 × 75 mL of ethyl acetate. The combined organic layers were washed with dilute brine until the aqueous washes were colorless (3–4 × 50 mL). The organic layer was dried over $MgSO_4$, filtered, and evaporated to a small volume (20–30 mL). Then hexane or petroleum ether (50–100 mL) was added in small portions. The mixture was then cooled to 0 °C to −20 °C to induce crystallization. The product was filtered onto scintered glass and dried in vacuo to yield the following N^{α}-formyl-α,β-dehydro amino acid ethyl esters.

Ethyl N^{α}-formyl-α-(1-ethylpropylidene)glycinate: mp 41.0–41.5 °C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.2 (d, 0.7 H, cis *H*CONH, 8.0–7.8 (d, 0.3 H, trans *H*CONH), 4.4–4.0 (q, 2 H, CO_2CH_2), 2.6–2.1 (m, 4 H, vinyl CH_2), 1.5–1.0 (m, 9 H, CH_3); mass spectrum, *m/e* 199 (M^+).

Ethyl N^{α}-formyl-α-cyclopentylideneglycinate: mp 93.0–94.0 °C; ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.2 (s, 0.8 H, cis *H*CONH), 8.1, 8.0 (d, 0.2 H, trans *H*CONH), 4.4–4.0 (q, 2 H, CO_2CH_2), 2.9–2.2 (m, 4 H, CH_2), 1.8–1.2 (m, 7 H, CH_2, CH_3); mass spectrum, *m/e* 197 (M^+).

Ethyl N^{α}-formyl-α-cyclohexylideneglycinate: mp 86.0–87.0 °C (lit.[13] mp 83.0–84.0 °C); ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.2 (s, 1 H, *H*CONH), 4.4–4.0 (q, 2 H, CO_2CH_2), 2.5–1.9 (br m, 10 H, CH_2), 1.3–1.0 (t, 3 H, CH_3); mass spectrum, *m/e* 211 (M^+).

Ethyl N^{α}-formyl-α-(4-*tert*-butylcyclohexylidene)glycinate: mp 96.5–97.0 °C; TLC R_f 0.25 (ethyl acetate/hexane, 1:1), R_f 0.45 (ethyl acetate/$CHCl_3$, 1:1); ^{1}H NMR (60 MHz, $CDCl_3$) δ 8.1 (d, 0.7 H, cis *H*CONH), 8.0– 7.8 (d, 0.3 H, trans *H*CONH), 4.4–4.0 (q, 2 H, CO_2CH_2), 3.7–3.4 (br t, 2 H, vinyl CH_2), 2.9–2.6 (br t, 2 H, vinyl CH_2), 2.1–1.7 (m, 3 H, CH_2, CH), 1.4–1.2 (t, 3 H, CH_3), 0.9 (s, 9 H, $C(CH_3)_3$); mass spectrum (free carboxylate), *m/e* 239 (M^+).

Preparation of *S*-*p*-Methylbenzyl-Protected α,β-Disubstituted Sulfhydryl Amino Acids 1b–3b. The N^{α}-formyl-α,β-dehydro amino acid ester (10–20 mmol) was dissolved in benzene (20–30 mL) at 27 °C. Phosphorus pentasulfide (P_4S_{10}, Aldrich Gold Label, 0.2 equiv) was added in one portion as a solid. The benzene was heated to reflux under a condenser that was protected from atmospheric moisture by a drying tube. The reaction was monitored by TLC in ethyl acetate/hexane (1:1). When TLC indicated that the reaction was complete, the solution was cooled to 27 °C and the benzene was decanted from the insoluble residue (or, more efficiently, filtered by gravity through no. 1 filter paper). The benzene was evaporated at reduced pressure to obtain the crude thiazoline as a clear yellow or red oil, which was used for the hydrolysis step without purification. The insoluble thiazoline was suspended in 4 N HCl (5–10 mL/mmol of thiazoline) and heated to gentle reflux under a condenser overnight. The homogeneous solution was cooled to 27 °C and extracted with ether (2 × 50 mL) until the organic extracts were colorless. The aqueous acid solution was evaporated under high vacuum to obtain the crude β,β-dialkylcysteine as the hydrochloride salt. The free sulfhydryl compounds **1a–3a** were difficult to crystallize and were used directly for the subsequent S-protection as crude products after drying over P_2O_5 in vacuo.

The sulfhydryl group of the β,β-dialkylcysteine derivatives was protected as the *p*-methylbenzyl thioether by the reaction of the crude hydrochloride cysteine salt (**1a**, **2a**, **3a**) with α-chloro-*p*-xylene in liquid ammonia/sodium at −78 °C.[15] This procedure

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was used to prepare **1b**, **2b**, and **3b**.

Ammonia (ca. 200 mL) was collected into a three-neck, 500-mL round-bottom flask fitted with a glass-covered stir bar and a dry ice/acetone condenser and protected from atmospheric moisture by a KOH trap. Metallic sodium was added in small pieces until a blue color persisted for 5 min at −78 °C. The vacuum-dried, crude sulfhydryl amino acid hydrochloride salt (5–10 mmol) was added in one portion; additional sodium was then added until a blue color persisted for 3 min. α-Chloro-*p*-xylene (1.5 equiv) was injected into the ammonia solution at −78 °C. the reaction mixture was stirred for 2 or 3 min at −78 °C, the dry ice/acetone bath was removed, and the bulk of the ammonia was evaporated under an air stream overnight. The solid residue was dissolved in water (10 mL/mmol), and the pH was adjusted to around 5.5 with acetic acid at 0 °C. This produced a voluminous white precipitate, which was filtered, washed with water (100 mL) and diethyl ether (200 mL), and dried in vacuo to obtain the zwitterion form of the *S*-*p*-methylbenzyl amino acids **1b**, **2b**, and **3b**. The crude products were recrystallized from methanol or methanol/water and dried over P_2O_5 in vacuo to obtain the *S*-*p*-methylbenzyl β,β-dialkylcysteines. ***S*-(*p*-Methylbenzyl)-β,β-diethylcysteine (1b)**: mp 156.0–157.0 °C; ninhydrin test positive; Ellman test negative; ^{1}H NMR (60 MHz, CF_3CO_2H/D_2O) δ 7.2 (s, 4 H, C_6H_4), 4.2 (s, 1 H, α-CH), 3.7 (s, 2 H, SCH_2), 2.4 (s, 3 H, $C_6H_4CH_3$), 2.0–1.8 (m, 4 H, CH_2), 1.2–1.0 (m, 6 H, CH_3). Anal. calcd for $C_{15}H_{23}NO_2S$: C, 55.33; H, 7.31; N, 4.61; S, 10.71. Found: C, 54.59; H, 7.65; N, 4.91; S, 10.33. ***S*-(*p*-Methylbenzyl)-β,β-tetramethylenecysteine (2b)**: mp 193.0–1.5.0 °C; ninhydrin test positive; Ellman test negative; ^{1}H NMR (250 MHz, CF_3CO_2H/Me_2SO-d_6) δ 8.3 (m, 3 H, NH_3), 7.2–7.1 (dd, 4 H, C_6H_4), 4.0 (br s, 1 H, α-CH), 3.6 (dd, 2 H, SCH_2), 2.3 (s, 3 H, $C_6H_4CH_3$), 2.0–1.5 (m, 8 H, CH_2). Anal. calcd for $C_{15}H_{21}NO_2S$: C, 64.47; H, 7.59; N, 5.01; S, 11.47. Found: C, 64.23; H, 7.44; N, 4.84; S, 11.26. ***S*-(*p*-Methylbenzyl)-β,β-pentamethylenecysteine (3b)**: mp 198.0–200.0 °C; ninhydrin test positive; Ellman test negative; ^{1}H NMR (250 MHz, CF_3CO_2H/Me_2SO-d_6) δ 7.3–7.1 (dd, 4 H, C_6H_4), 3.9 (br s, 1 H, α-CH), 3.7 (dd, 2 H, SCH_2, 2.3 (s, 3 H, $C_6H_4CH_3$), 2.0–1.5 (m, 8 H, CH_2), 1.2 (m, 2 H, CH_2). Anal. calcd for $C_{16}H_{23}NO_2S$: C, 65.48; H, 7.92; N, 4.77; S, 10.92. Found: C, 65.43; H, 7.95; N, 4.64; S, 10.64.

***S*-(*p*-Methylbenzyl)-β,β-(3-*tert*-butylpentamethylene)cysteine (4b).** The dehydro ester ethyl N^{α}-formyl-α-(4-*tert*-butylcyclohexylidene)glycinate (3.5 g, 13 mmol) was dissolved in benzene (50 mL) and stirred at 27 °C. Phosphorus pentasulfide (1.35 g, 3 mmol) was added in one portion, and the mixture was heated under a reflux condenser for 1 h. The benzene was filtered by gravity (no. 1-type filter paper) and evaporated at reduced pressure. The insoluble residual oil was suspended in 4 N HCl (50 mL) and heated to gentle reflux. After 2 days, the heating was stopped, the mixture was cooled to 27 °C, and the solid was filtered and washed with water (100 mL) and diethyl ether (100 mL). A ^{1}H NMR spectrum ($CDCl_3/CF_3CO_2H$) showed that the hydrolysis was 70–80% complete, as measured by the integration of the ethyl ester signals. The crude product was taken in 4 N HCl (100 mL) and refluxed for an additional 48 h. The white solid was filtered and washed with water and diethyl ether. The product was dried in vacuo over P_2O_5 to obtain β,β-(3-*tert*-butylpentamethylene)cysteine hydrochloride as a white solid (2.2 g, 60%): ninhydrin test positive; Ellman test negative; ^{1}H NMR (60 MHz, Me_2SO-d_6/CF_3CO_2H) δ 3.9 (s, 1 H, α-CH), 2.0–1.3 (m, 9 H, CH_2, CH), 0.80 (s, 9 H, $C(CH_3)_3$); mass spectrum, *m/e* 280 (M^+). Approximately 200 mL of liquid ammonia was collected into a three-neck, 500-mL round-bottom flask fitted with a glass stir bar and a dry ice/acetone condenser and protected from atmospheric moisture by a KOH trap. Metallic sodium was added in small pieces until a blue color persisted for 5 min. Then β,β-(3-*tert*-butylpentamethylene)cysteine hydrochloride (2.1 g, 10 mmol) was added in one portion, followed by dry THF (30 mL), which dissolved most of the solid. Additional sodium was then added until a blue color persisted for 3 min. Then α-chloro-*p*-xylene (2.0 mL, 14 mmol) was injected into the NH_3/THF solution, and the mixture was stirred for 2–3 min longer, during which time the blue color dissipated. The dry ice/acetone bath was removed, and the NH_3/THF was removed overnight by blowing air through the flask. The solid was filtered, washed with water (200 mL) and ether (200 mL), and dried in vacuo. The crude *S*-(*p*-methylbenzyl)-β,β-(3-*tert*-butylpentamethylene)cysteine was recrystallized from methanol/water and dried in vacuo to obtain pure **4b** (2.2 g, 68%): mp 177.5–180.0 °C; ninhydrin test positive; Ellman test negative; ^{1}H NMR (CF_3CO_2H/Me_2SO-d_6) δ 7.2 (m, 4 H, C_6H_4), 3.9 (s, 1 H, α-CH), 3.7 (m, 2 H, SCH_2), 2.4 (s, 3 H, $C_6H_4CH_3$), 2.0–1.3 (m, 9 H, CH_2, CH), 0.80 (s, 9 H, $C(CH_3)_3$). Anal. calcd for $C_{20}H_{31}NO_2S$: C, 68.71; H, 8.96; N, 4.01; S, 9.17. Found: C, 68.77; H, 8.62; N, 3.90; S, 8.60.

Preparation of N^{α}-*t*-Boc Derivatives of the *S*-(*p*-Methylbenzyl)-β,β-disubstituted Sufhydryl Amino Acids To Give 1c, 2c, 3c, and 4c. The α-amino groups of the above amino acid derivatives were protected by reactions of the *S*-*p*-methylbenzyl-β,β-dialkylcysteine derivatives **1b**, **2b**, **3b**, or **4b** with di-*tert*-butyl dicarbonate in aqueous dioxane at pH 10.5, following procedures similar to those previously reported.[16,17] The following general method was used. The *S*-*p*-methylbenzyl-β,β-disubstituted cysteine analogues (**1b**, **2b**, **3b**, or **4b**; 2.3 mmol) were suspended in 50 mL of dioxane/water (2:1), and the pH was adjusted to 10.5 with 1 N NaOH. Di-*tert*-butyl dicarbonate (650 mg, 3 mmol) was added as a solution in ca. 2 mL of dioxane via a pipet. The pH was maintained at pH 10.5 by periodic addition of 1 N NaOH solution, until no further decrease in pH was observed. The dioxane was removed by vacuum evaporation, ethyl acetate (75 mL) was added, and the mixture was cooled to 0 °C and acidified to pH 2.1 with cold 4% HCl. The aqueous layer was extracted rapidly with the ethyl acetate, followed by a second 50-mL extraction with ethyl acetate. The combined organic extracts were washed with 4% HCl (2 × 40 mL) and water (3 × 75 mL), dried, filtered, and evaporated, to obtain the crude **4c** as a clear, colorless oil, in 70% yield. The crude product was recrystallized from dichloromethane/hexane at −20 °C, filtered, and dried over P_2O_5 in vacuo to yield the final product.

***N*$^{\alpha}$-*t*-Boc-*S*-(*p*-methylbenzyl)-β,β-diethylcysteine (1c)**: mp 91.0–92.0 °C; ninhydrin test negative; Ellman test negative; TLC R_f 0.20 (ethyl acetate/$CHCl_3$, 1:1), R_f 0.40 ($CHCl_3/CH_3OH$, 9:1); ^{1}H NMR (60 MHz, $CDCl_3$) δ 7.2 (s, 4 H, C_6H_4), 5.8 (br s, 1 H, NH), 4.5 (d, 1 H, α-CH), 3.7 (br s, 2 H, SCH_2), 2.4 (s, 3 H, $C_6H_4CH_3$), 2.0–1.5 (m, 4 H, CH_2), 1.45 (s, 9 H, $C(CH_3)_3$), 1.1–1.0 (m, 6 H, CH_3). Anal. calcd for $C_{20}H_{31}NO_4S \cdot {}^1/_2H_2O$: C, 61.50; H, 8.29; N, 3.59; S, 8.21. Found: C, 61.32; H, 8.49; N, 3.70; S, 8.49.

***N*$^{\alpha}$-*t*-Boc-*S*-(*p*-methylbenzyl)-β,β-diethylcysteine (2c)**: mp 57.0–58.5 °C; ninhydrin test negative; Ellman test negative; TLC R_f 0.14 ($CHCl_3/CH_3OH$, 9:1), R_f 0.47 (acetone/acetic acid, 98:2); ^{1}H NMR (60 MHz, $CDCl_3$) δ 7.2–7.0 (m, 4 H, C_6H_4), 5.5 (br d, 1 H, NH), 4.35 (br d, 1 H, α-CH), 3.6 (br s, 2 H, SCH_2), 2.3 (s, 3 H, $C_6H_4CH_3$), 2.0–1.6 (m, 8 H, CH_2), 1.45 (s, 9 H, $C(CH_3)_3$). Anal. Calcd for $C_{20}H_{29}NO_4S$: C, 57.80; H, 8.02; N, 3.37; S, 7.71. Found: C, 57.60; H, 7.74; N, 3.62; S, 7.11.

***N*$^{\alpha}$-*t*-Boc-*S*-(*p*-methylbenzyl)-β,β-pentamethylenecysteine (3c)**: mp 123.0–124.0 °C; ninhydrin test negative; Ellman test negative; TLC R_f 0.14 (ethyl acetate/$CHCl_3$, 1:1), R_f 0.34 ($CHCl_3/CH_3OH$, 9:1); ^{1}H NMR (60 MHz, $CDCl_3$) δ 7.2 (m, 4 H, C_6H_4), 5.7 (br s, 1 H, NH), 4.4 (d, 1 H, α-CH), 3.6 (s, 2 H, SCH_2), 2.4 (s, 3 H, $C_6H_4CH_3$), 2.0–1.4 (m, 19 H, CH_2, $C(CH_3)_3$). Anal. calcd for $C_{21}H_{31}NO_4S$: C, 64.08; H, 7.95; N, 3.56; S, 8.14. Found: C, 63.84; H, 8.22; N, 3.48; S, 7.84.

***N*$^{\alpha}$-*t*-Boc-*S*-(*p*-methylbenzyl)-β,β-(3-*tert*-butylpentamethylene)cysteine (4c)**: mp 92.0–94.0 °C; ninhydrin test negative; Ellman test negative; ^{1}H NMR (250 MHz, $CDCl_3$) δ 7.2–6.9 (m, 4 H, C_6H_4), 5.7 (d, 1 H, NH), 4.3 (d, 1 H, α-CH), 3.7 (d, 2 H, SCH_2), 2.4 (s, 3 H, $C_6H_4CH_3$), 1.8–1.3 (m, 18 H, CH_2, CH, $C(CH_3)_3$). Anal. Calcd for $C_{25}H_{39}NO_4S$: C, 65.85; H, 9.00; N, 3.20; S, 7.13. Found: C, 66.32; H, 8.70; N, 3.14; S, 6.72.

Acknowledgment. Supported by National Science Foundation Grant PCM-8412084.