thesis. In the final cycles of weighted least-squares refinement, 361 parameters were refined including the overall scale factor, position parameters, and anisotropic thermal parameters for all nonhydrogen atoms. The hydrogen atoms were placed at ideal positions and distances[14] and given arbitrary isotropic temperature parameters[15] (B = 4.0 Å^2). Convergence was achieved with R_1 = 0.054 and R_2 = 0.33.[16] In the last cycle of refinement, the maximum shift per error was 0.246 and the average shift per error was 0.055. The highest peak on a final difference electron density map represented less than 0.39 e Å^{-3}. Neutral atom scattering factors were used for Br, P, O, N, C,[17] and H[18] and corrected for anomalous dispersion.[19]

Registry No. 4a, 79839-11-5; **4b**, 79839-12-6; (±)-**5**, 79839-13-7; **6**, 68374-69-6; **7**, 79839-14-8; *trans*-cinnamaldehyde, 14371-10-9; benzaldehyde, 100-52-7.

Supplementary Material Available: Tables B-F, nonhydrogen atomic positional parameters, hydrogen atomic positional parameters, anisotropic thermal parameters, bond lengths, and bond angles (5 pages). Ordering information is given on any current masthead page.

(14) Hydrogen atoms were placed at 0.97 Å from the atoms to which they bond with the expected geometry.
(15) Isotropic thermal parameters were of the form: $\exp(-B \sin^2\theta/\lambda^2)$.
(16) $R_1 = \sum||F| - |F_c||/\sum|F_o|$; $R_2 = [\sum w(|F_o| - |F_c|)^2/w(F_o)^2]^{1/2}$.
(17) Cromer, D. T.; Mann, J. B. *Acta Crystallogr., Sect. A* **1968**, *24*, 321.
(18) Stewart, R. F.; Davidson, E. R.; Simpson, W. T. *J. Chem. Phys.* **1965**, *42*, 3175.
(19) "International Tables for X-Ray Crystallography", 3rd ed.; Kynoch Press: Birmingham, England, 1968; Vol. 3, p 215.

Protected Lactam-Bridged Dipeptides for Use as Conformational Constraints in Peptides

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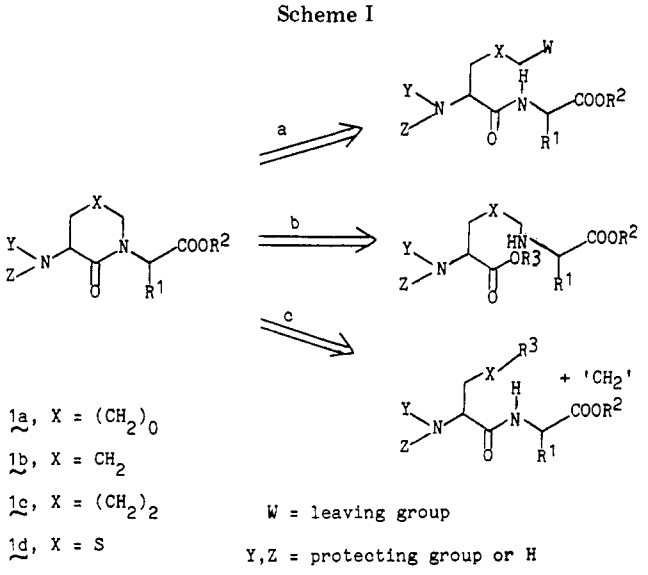
General methods have been devised for the synthesis of lactam-constrained dipeptide analogues having five-, six-, and seven-membered rings. Three different paths from protected chiral α-amino acids to lactams involving intramolecular alkylation, intramolecular acylation, and insertion of a one carbon unit by condensation with formaldehyde have been utilized. The first two methods produce chiral products stereospecifically, but considerable racemization occurs in the third route which leads to a 4-oxo-5-amino-1,3-thiazine (**13**). The products are prepared in good yield and have protecting groups making them suitable for incorporation into higher peptides by methods commonly used.

Lactams have been shown to be a useful new type of conformational constraint in peptides. Information may be obtained about the bioactive conformation of a peptide, and biological potency may be increased by incorporation of a lactam.[1] Not only does this restriction fix the trans peptide bond but it also introduces constraints which limit conformation by noncovalent interactions. Ring size of the lactam has been shown to have an important effect on conformation and also on biological potency of an analogue. In a comparison of rumen methane inhibiting analogues differing only in lactam ring size (five, six, or seven membered), only the six-membered ring showed high activity.[2] The lactam constraint can also be useful synthetically by allowing construction of cyclic hexapeptide analogues through cyclotrimerization.[3] We report here the synthesis of γ-, δ-, and ε-lactam-bridged dipeptides **1**, including 4-oxo-1,3-thiazines **1d** which have been used as peptide conformational constraints.

Several criteria had to be met in order for the use of compounds **1** in peptide synthesis to be practical. These structures should be obtainable in a small number of steps and in a protected form. Protecting groups should be of a type that allows incorporation of the lactams into peptides by using standard procedures. The lactams preferably should be obtained optically pure from chiral starting materials.

Scheme I



We have utilized three different synthetic routes from α-amino acids to the target lactam structures (illustrated retrosynthetically in Scheme I). These approaches involve

(1) (a) Freidinger, R. M.; Veber, D. F.; Perlow, D. S.; Brooks, J. R.; Saperstein, R. *Science* **1980**, *210*, 656. (b) Freidinger, R. M. In "Peptides: Proceedings of the 7th American Peptide Symposium"; Rich D., Ed.; Pierce Chemical Co.: Rockford, IL, in press.
(2) Freidinger, R. M.; Veber, D. F.; Hirschmann, R.; Paege, L. M. *Int. J. Pept. Protein Res.* **1980**, *16*, 464.
(3) Freidinger, R. M.; Schwenk, D. A.; Veber, D. F. In "Peptides: Proceedings of the 6th American Peptide Symposium"; Gross, E., Meienhofer, J., Eds.; Pierce Chemical Co.: Rockford, IL, 1979; p 703.

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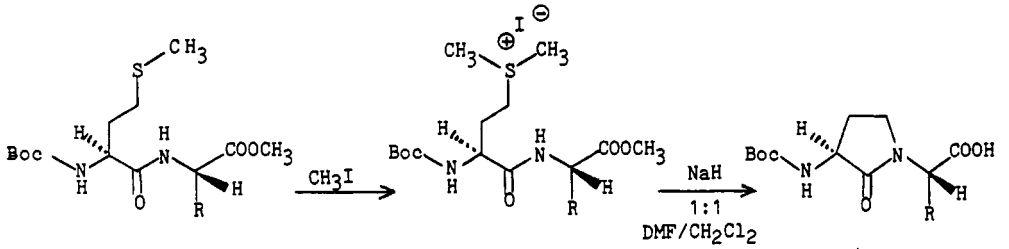
Scheme II



2a, R = H
b, R = $CH_2CH(CH_3)_2$
c, R = CH_2Ph
d, R = $CH_2CH_2CH_2CH_2$-NHCbz

3

4

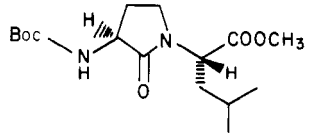
intramolecular alkylation (path a), intramolecular acylation (path b), and insertion of a one-carbon unit by condensation with formaldehyde (path c) to form the lactam ring.

Each of these paths meet the criteria set forth except that some racemization occurs in path c. Protected common amino acids in optically pure form have served as starting materials in order to define the chirality of the products.

Results and Discussion

An intramolecular alkylation route (Scheme I, path a) was applied to the synthesis of γ-lactam dipeptides (Scheme II). Protected methionine dipeptides were chosen as starting materials since the methylthio function can potentially be converted into a leaving group. These dipeptides were prepared by standard methods from (*tert*-butoxycarbonyl)methionine and amino acid methyl esters, both in optically pure form (D or L). Sulfonium salts **3** were formed by the action of methyl iodide, and cyclization to the lactams **4** was induced by sodium hydride in 1:1 DMF/methylene chloride in 40–60% yield.[4] Of the other bases investigated, only lithium diisopropylamide (LDA) showed promise. Diazabicycloundecene (DBU) in THF demethylated the sulfonium salt in good yield, and no product was isolated from aqueous sodium hydroxide treatment. This is the first observation of an intramolecular N-alkylation of a methionine sulfonium salt. Normally, the cyclization of these intermediates results in amide O-alkylation and is used extensively for peptide degradation and sequence studies.[5] In this case, generation of the amide anion with strong base changes the course of the reaction. An intramolecular C-alkylation of methionine sulfonium salts has also been reported.[6]

Surprisingly, the methyl ester is almost completely hydrolyzed under the conditions of the cyclization. The major isolated product is the acid **4** with only small amounts of the corresponding methyl ester being found. In our examples, this hydrolysis was fortuitous since the next synthetic step would have been saponification of the ester. The source of this ester cleavage was briefly investigated with methyl ester **5**.[7] Sodium iodide and dimethyl sulfide in 1:1 DMF–CH_2Cl_2 at 0 °C gave no change. Addition of a 50% mineral oil suspension of NaH, however, gave a rapid conversion to acid ($t_{1/2} \approx 5$ min). This change is comparable to the rate of disappearance of a transient TLC spot of higher R_f observed in the cyclization reactions. Powdered NaOH added to the NaI–dimethyl sulfide mixture also effected the conversion, but at a slower rate ($t_{1/2} \approx 30$ min). It is likely that the saponification in the lactam-forming cyclizations is due to Na_2O contaminating the NaH. Additional support for this explanation comes from the observation that cyclization with LDA produces ester rather than acid.



5

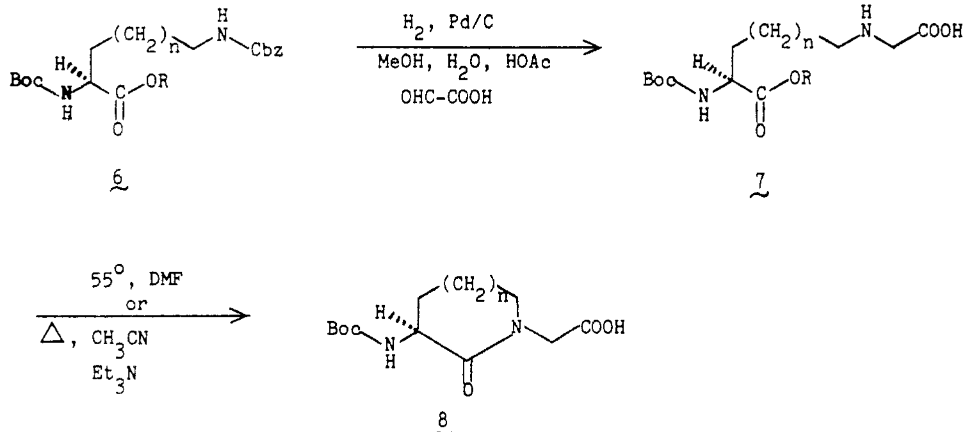
This synthetic route provides ready access to a variety of lactams **4** of defined stereochemistry simply by changing the starting amino acid ester. Epimerization at the asymmetric centers is normally not observed although, in the case of **4c**, NMR analysis revealed that 12–15% racemization occurred in the phenylalanine-derived portion. Strongly basic protic conditions during workup cause extensive epimerization and should be avoided. This route is also potentially applicable to the methionine sulfonium salts of larger peptides. As an example, the sulfonium salt of Boc-D-Ala-Met-Phe-OMe was cyclized to the expected lactam tripeptide.

The δ- and ε-lactams were prepared according to Scheme III by an intramolecular acylation route (Scheme I, path b) since the two homologues of methionine required for path a are not readily available. The commercially available ornithine and lysine derivatives **6** were hydrogenolyzed over palladium–carbon catalyst to remove the side-chain protection followed by reductive alkylation in situ with glyoxylic acid. This reaction was monitored by TLC and stopped when the monoalkylation product **7** was predominant. Diacid **7a** was obtained in 54% yield as a crystalline solid which was indefinitely stable at −20 °C. The compound cyclized to **8a** slowly in DMF at room temperature and rapidly at 55 °C without carboxyl activation. Cyclization was also observed in methanol at a reduced rate. No lactam formation was observed in water at 60 °C under acidic, basic, or neutral conditions, and compound **7a** was recovered unchanged. This failure to cyclize does not represent an unfavorable equilibrium since **8a** does not revert to **7a** under these conditions. Removal of the Boc protection with HCl in ethyl acetate at 0 °C and analysis of the resultant compound by the method of Manning and Moore[8] (using the *N*-carboxy anhydride of leucine for derivatization) demonstrated the optical purity.

Formation of the seven-membered ring was considerably more difficult. No cyclization occurred on heating the

(4) Similar conditions have been used to prepare β-lactams from β-chloro amides. Cf.: Baldwin, J. E.; Au, A.; Christie, M.; Haber, S. B.; Hesson, D. *J. Am. Chem. Soc.* **1975**, *97* 5957.
(5) Gross, E.; Witkop, B. *J. Biol. Chem.* **1962**, *237*, 1856.
(6) Rich, D. H.; Tam, J. P. *Synthesis* **1978**, 46.
(7) Ester **5** was prepared from the corresponding acid by using the DBU–MeI method. Cf.: Rao, C. G. *Org. Prep. Proc. Int.* **1980**, *12*, 225.
(8) Manning, J. M.; Moore, S. *J. Biol. Chem.* **1968**, *243*, 5591.

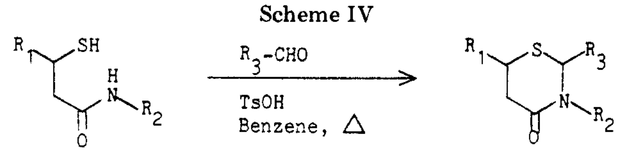
Scheme III



6 7 8

a, R = H, n = 1
b, R = H, n = 2
c, R = CH_3, n = 2

Scheme IV



10 9

crude diacid **7b** in DMF or other solvents. Thermal gravimetric analysis showed that a weight loss corresponding to CO_2 and isobutylene, the elements of the Boc group, eventually occurs at 200 °C, but cyclization is not seen. Attempts using activating agents such as dicyclohexylcarbodiimide/*N*-hydroxysuccinimide or triphenylphosphine/carbon tetrachloride were also unsuccessful. The half-acid ester **7c** was found to cyclize slowly in refluxing acetonitrile in the presence of triethylamine. The overall yield from starting N^α-Boc-N^ϵ-Cbz-Lys methyl ester[9] was 25%, and no racemization was observed.

This intramolecular acylation route could potentially be extended to the synthesis of δ-lactams **8** other than X-Gly derivatives. Introduction of side chains on the glycine unit would require the use of pyruvic acid derivatives which have limited availability. Furthermore, the resultant asymmetric carbon would be present in both D and L forms, thus requiring a separation of diastereomers.

The 4-oxo-5-amino-1,3-thiazine system was investigated because it potentially provides access to a broader range of δ-lactam dipeptides. This heterocycle was prepared by path c (Scheme I) based on the report of Kametani, who showed that simple 4-oxo-1,3-thiazines **9** could be made from β-thio amides **10** and aldehydes in the presence of a strong acid catalyst (Scheme IV).[10]

The starting material for our lactam synthesis was N^α-phthalyl-*S*-(acetamidomethyl)-(*R*)-cysteine (**11**), which was prepared from Acm-(*R*)-Cys according to the phthalylation procedure of Nefkens.[11] It was considered necessary to use the phthalimide protecting group in order to preclude cyclization to a thiaproline derivative under the Kametani reaction conditions. Acylation of glycine methyl ester by compound **11** with dicyclohexylcarbodiimide-1-hydroxybenzotriazole or preferably diphenylphosphorylazide provided dipeptide **12** (Scheme V).

Removal of the Acm protecting group with mercuric acetate[12] gave the crude thiol which was cyclized without purification to the desired lactam **13** in 25–40% yield. It was subsequently discovered that compound **13** can be prepared directly from dipeptide **12** by heating this compound with paraformaldehyde and *p*-toluenesulfonic acid in 1,1,2-trichloroethane with azeotropic water removal. Indeed, crystalline lactam **13** was obtained in 56% yield by this more direct route. When the reaction was repeated without the paraformaldehyde, no product was formed. The formaldehyde is, therefore, essential for the reaction and must function as an electrophile assisting removal of the Acm group as well as furnishing the methylene group to the complete the cyclization. Initial attack of either sulfur or amide nitrogen on formaldehyde will lead to an intermediate which could cyclize followed by loss of the Acm group. Alternatively, Acm loss may occur after addition of sulfur to formaldehyde but prior to cyclization. This novel in situ deblocking of cysteine sulfur is reminiscent of the iodine oxidation of Acm-Cys peptides.[13] Wolfe has synthesized heterocycles such as **13** from dipeptide sulfoxides by way of the Pummerer rearrangement,[14] and Young has prepared the 3-acetyl heterocycle by cyclization of N^α-Boc-*S*-Acm-Cys with dicyclohexyl carbodiimide–1-hydroxybenzotriazole.[15]

For utilization of intermediate **13** in peptide synthesis, selective removal of one of the protecting groups was necessary. Selective hydrolysis of the methyl ester was accomplished by using 4% HCl in refluxing aqueous THF, a modification of a method described by Sheehan.[16] Little, if any, hydrolysis of the thiazinone system occurred, and acid **14** was isolated in 85% yield.

The most serious limitation of this method presently is racemization at the cysteine α-carbon under the cyclization

(9) Compound **6c** was prepared by using the cesium salt method. Cf.: Wang, S. S.; Gisin, B. F.; Winter, D. P.; Makofske, R.; Kulesha, I. D.; Tzougraki, C.; Meienhofer, J. *J. Org. Chem.* **1977**, *42*, 1286.

(10) Kametani, T.; Kigasawa, K.; Hiiragi, M.; Wagatsuma, N.; Kohagizawa, T.; Inoue, H. *Heterocycles* **1978**, *9*, 831.

(11) Nefkens, G. H. L.; Tesser, G. I.; Nivard, R. J. F. *Recl. Trav. Chim. Pays-Bas* **1960**, *79*, 688.

(12) Veber, D. F.; Milkowski, J. D.; Varga, S. L.; Denkewalter, R. G.; Hirschmann, R. *J. Am. Chem. Soc.* **1972**, *94*, 5456.

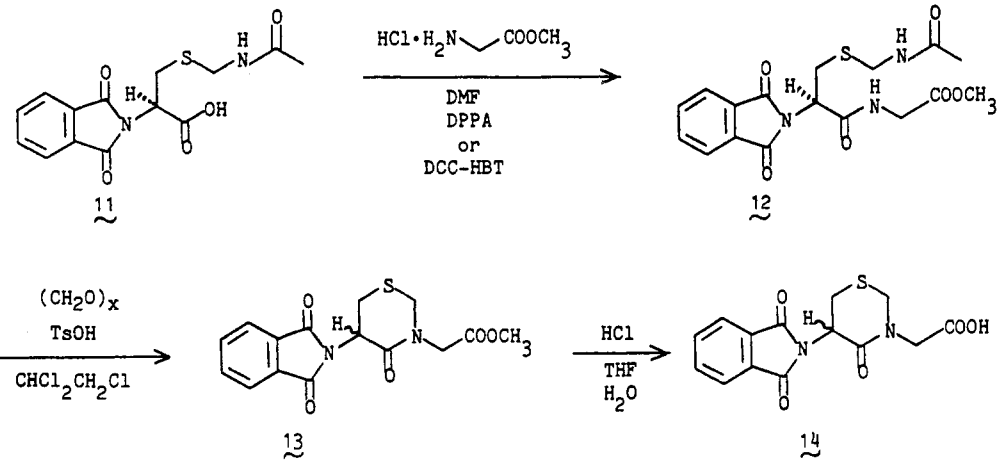
(13) Kamber, B.; Hartmann, A.; Eisler, K.; Riniker, B.; Rink, H.; Sieber, P.; Rittel, W. *Helv. Chim. Acta* **1980**, *63*, 899.

(14) Wolfe, S.; Kazmaier, P. M.; Auksi, H. *Can. J. Chem.* **1979**, *57*, 2412.

(15) Wiliams, B. J.; Young, G. T. In "Peptides: Proceedings of the 6th American Peptide Symposium"; Gross, E., Meienhofer, J., Eds.; Pierce Chemical Co.: Rockford, IL, Illinois, 1979; p 321.

(16) Sheehan, J. C.; Goodman, M.; Hess, G. P. *J. Am. Chem. Soc.* **1956**, *78*, 1367.

Scheme V



conditions. NMR analysis, with a chiral shift reagent, of compound **13** derived from (*R*)-cysteine showed a 2:1 mixture. Optimization of reaction conditions could potentially decrease this problem. This method can potentially provide a variety of analogues of **14** for use as conformational constraints in peptides by variation of the amino acid methyl ester and aldehyde components. Kametani has already demonstrated the successful use of several aldehydes.[10]

Conclusion

General schemes have been devised for the synthesis of lactam-constrained dipeptide analogues having five-, six-, and seven-membered rings. The method for preparation of five-membered lactam analogues allows the introduction of two asymmetric centers from optically pure starting materials with little or no racemization. Stereo control of one asymmetric center is possible in the scheme for synthesis of six- and seven-membered lactams. A synthetic route to a 4-oxo-5-amino-1,3-thiazine which allows introduction of two asymmetric centers shows racemization at the center in the lactam ring during the synthesis. The analogues are prepared in good yield and have protecting groups making them suitable for incorporation into higher peptides by methods commonly used.

Experimental Section[17]

Capillary melting points were determined on a Thomas-Hoover apparatus and are uncorrected. NMR spectra were recorded on a Varian EM-390 spectrometer and are expressed in parts per million from Me_4Si as internal standard. Infrared spectra were recorded on a Perkin-Elmer 137 spectrophotometer. Optical rotations were recorded on a Perkin-Elmer 141 polarimeter. Thin-layer chromatography (TLC) was performed on silica gel (Quantam Industries, Q-1 plates), and the components were visualized either by *tert*-butyl hypochlorite–KI reagents, by ninhydrin, or by iodoplatinate. Systems used in TLC were as follows: A, 5:5:1:3 ethyl acetate–pyridine–acetic acid–water (EPAW); B, 10:5:1:3 EPAW; C, 90:10:1 chloroform–methanol–water (CMW); D, 30:5:1:1 EPAW; E, 20:5:1:1 EPAW; F, 60:5:1:1 EPAW.

Boc-Met-Gly Methyl Ester (2a). (*tert*-Butoxycarbonyl)-methionine (24.9 g, 0.1 mol) and glycine methyl ester hydrochloride (12.6 g, 0.1 mol) were mixed in 150 mL of degassed DMF. Triethylamine (13.9 mL, 0.1 mol) and HBT (15.3 g, 0.1 mol) were dissolved in the mixture, and DCC (20.6 g, 0.1 mol) was added. The mixture was stirred at room temperature overnight and filtered. The filtrate was concentrated in vacuo, and the residue was redissolved in methylene chloride (150 mL). This solution was washed with 0.5 M citric acid (3 × 50 mL) and 2 N aqueous $NaHCO_3$ (3 × 50 mL) and dried over Na_2SO_4. Filtration and concentration in vacuo gave a crude product which crystallized. Recrystallization from ethyl acetate–hexane gave **2a** in three crops of crystals: 24.1 g (75%); mp 87–89 °C; IR (CH_2Cl_2) 3400, 2950, 1745, 1710, 1680, 1490, 1360, 1165 cm^{-1}; NMR ($CDCl_3$) δ 1.43 (s, 9), 2.08 (s over m, 5), 2.58 (t, 2, J = 7 Hz), 3.70 (s, 3), 4.00 (d, 2, J = 6 Hz), 4.28 (m, 1), 5.27 (d, 1, J = 8 Hz), 6.78 (br t, 1, J = 6 Hz); $[\alpha]^{24}_D$ −20.59° (*c* 0.96, MeOH).

Anal. Calcd for $C_{13}H_{24}N_2O_5S$: C, 48.73; H, 7.55; N, 8.74. Found: C, 48.88; H, 7.79; N, 8.95.

Boc-Met-Leu Methyl Ester (2b). This compound was prepared on a 50-mmol scale by using the procedure described for **2a** except that HBT was omitted and the reaction solvent was methylene chloride. Crystallization from ethyl acetate–hexane gave **2b** in three crops: 15.1 g (80%); mp 108–109 °C; NMR ($CDCl_3$) δ 0.93 (d, 6, J = 5 Hz), 1.44 (s, 9), 2.11 (s, 3), 1.5–2.0 (m, 5), 2.61 (t, 2, J = 7.5 Hz), 3.74 (s, 3), 4.37 (m, 1), 4.63 (m, 1), 5.23 (d, 1, J = 8 Hz), 6.62 (d, 1, J = 8 Hz); $[\alpha]^{24}_D$ −37.1° (*c* 1.0, MeOH).

Anal. Calcd for $C_{17}H_{32}N_2O_5S$: C, 54.23; H, 8.57; N, 7.44. Found: C, 54.00; H, 8.66; N, 7.38.

Boc-Met-Phe Methyl Ester (2c). This compound was prepared on a 56-mmol scale by using the procedure described for **2b**. Recrystallization from ethyl acetate–hexane gave **2c** in three crops: 11.9 g (52%); mp 83–85 °C; IR (CH_2Cl_2) 3400, 2920, 1730, 1700, 1675, 1480, 1350, 1160 cm^{-1}; NMR ($CDCl_3$) δ 1.45 (s, 9), 2.07 (s, 3), 1.8–2.2 (m, 2), 2.55 (t, 2, J = 7.5 Hz), 3.14 (d, 2, J = 6 Hz), 3.71 (s, 3), 4.28 (m, 1), 4.88 (m, 1), 5.15 (d, 1, J = 8.5 Hz), 6.63 (d, 1, J = 6.5 Hz), 7.1–7.4 (m, 5); $[\alpha]^{24}_D$ −14.2° (*c* 1.0, EtOH); amino acid analysis (20 h hydrolysis): $Met_{0.96}$-$Phe_{1.04}$.

Boc-Met-ε-Cbz-Lys Methyl Ester (2d). (*tert*-Butoxycarbonyl)methionine (8.4 g, 33.7 mmol) and ε-(benzyloxycarbonyl)lysine methyl ester hydrochloride (11.7 g, 33.7 mmol) were dissolved in 150 mL of degassed DMF, and triethylamine (4.7 mL, 33.7 mmol) was added. The mixture was cooled in an ice–water bath, and DPPA (7.3 mL, 33.7 mmol) was added. The reaction mixture was stirred at 0 °C for 1.5 h and then allowed to warm slowly to room temperature. Additional triethylamine (7 mL) was added in aliquots over a 2.5-h period to maintain a pH of 7.5. The mixture was stirred at room temperature overnight followed by concentration to a small volume. The residue was partitioned between ethyl acetate (200 mL) and 0.5 M aqueous citric acid (100 mL). The organic layer was washed further with aqueous citric acid (2 × 100 mL), 1 N $NaHCO_3$ (2 × 100 mL), and saturated aqueous NaCl (100 mL). The ethyl acetate layer was then dried (Na_2SO_4) and concentrated in vacuo to a viscous oil (18.7 g, 109%). A sample crystallized when the mixture was allowed to stand: mp 74–76 °C: NMR ($CDCl_3$) δ 1.43 (s, 9), 1.2–2.1 (m, 8), 2.07 (s, 3), 2.54 (t, 2, J = 7 Hz), 3.17 (m, 2), 3.73 (s, 3), 4.1–4.8 (m, 2), 5.10 (s plus m, 3), 6.83 (br d, 1, J = 8 Hz), 7.37 (s, 5); TLC R_f 0.38 (98:2 $CHCl_3$–MeOH).

Boc-D-Ala-Met-Phe Methyl Ester. Boc-D-Ala-Met-Phe-O-resin (7 mmol) was prepared by solid phase synthesis from

(17) Abbreviations: Boc, *tert*-butoxycarbonyl; DMF, dimethylformamide; THF, tetrahydrofuran; Cbz, (benzyloxy)carbonyl; Acm, acetamidomethyl; DPPA, diphenylphosphoryl azide; DCC, dicyclohexyl carbodiimide; HBT, 1-hydroxybenzotriazole; Ts, *p*-toluenesulfonyl; Me_2SO, dimethyl sulfoxide. Amino acid abbreviations follow the IUPAC–IUB convention in: *Pure Appl. Chem.* **1974**, *40*, 291.

Boc-Phe-O-resin.[18] Coupling was monitored with the Kaiser test,[19] and recoupling was required at each step. Ethanedithiol was added to the 1:1 trifluoroacetic acid–methylene chloride deblockings after introduction of methionine.

The tripeptide resin was suspended in methanol (140 mL) with triethylamine (14 mL) and stirred at room temperature for 2 h. The resin was filtered and retreated with Et_3N–MeOH for 24 h. The filtrates from the two batches were comparable by TLC (system C) and were combined and concentrated in vacuo. The residue crystallized and was filtered and washed with 1:1 ethylacetate–hexane to give 1.53 g (47%) of product in two crops: mp 65–66 °C; NMR (CD_3OD) δ 1.27 (d, 3, J = 7.0 Hz), 1.43 (s, 9), 1.95 (m, 2), 2.03 (s, 3), 2.35–2.6 (m, 2), 3.0–3.17 (m, 2), 3.67 (s, 3), 4.02 (q, 1, J = 7 Hz), 4.4–4.8 (m, 2), 7.23 (s, 5).

Boc-Met-Gly Methyl Ester Methylsulfonium Iodide (3a). [(*tert*-Butoxycarbonyl)methionyl]glycine methyl ester (960 mg, 3 mmol) was dissolved with stirring in 6 mL of methyl iodide at room temperature. A gummy solid separated over a period of 6.5 h. The supernatant was drawn off, and the residue was dried in vacuo to a hygroscopic foam: 1.41 g (100%); NMR ($CDCl_3$) δ 1.42 (s, 9), 2.48 (m, 2), 3.23 (s, 3), 3.32 (s, 3), 3.70 (s, 3 over m, 2), 4.00 (d, 2, J = 5.5 Hz), 4.47 (m, 1), 6.00 (d, 1, J = 7.5 Hz), 8.03 (m, 1).

Boc-Met-Leu Methyl Ester Methylsulfonium Iodide (3b). Boc-Met-Leu-OMe (8 g, 21.3 mmol) in methyl iodide for 2 days gave 11 g (100%) of sulfonium salt **3b** as a syrup after removal of methyl iodide in vacuo and three evaporations from methylene chloride solution: NMR ($CDCl_3$) δ 0.93 (m, 6), 1.43 (s, 9), 1.6–1.9 (m, 3), 2.3–2.7 (m, 2), 3.20 (s, 3), 3.33 (s, 3), 3.70 (s, 3), 3.55–4.0 (m, 2), 4.4–4.7 (m, 2), 5.9 (d, 1, J = 7 Hz), 8.1 (d, 1, J = 7.5 Hz).

Boc-Met-Phe Methyl Ester Methylsulfonium Iodide (3c). Boc-Met-Phe-OMe (11.9 g, 29 mmol) in methyl iodide (70 mL) gave sulfonium salt **3c** as a white precipitate: 15.6 g (98%): NMR ($CDCl_3$) δ 1.40 (s, 9), 2.2–2.6 (m, 2), 3.17 (s, 3), 3.30 (s, 3), 3.69 (s, 3), 3.0–3.9 (m, 4), 4.48 (m, 1), 4.75 (m, 1), 5.9 (d, 1, J = 7.5 Hz), 7.3 (s, 5), 7.95 (d, 1, J = 7.5 Hz).

Boc-Met-ε-Cbz-Lys Methyl Ester Methylsulfonium Iodide (3d). Boc-Met-Lys(ε-Cbz)-OMe (18.7 g) was dissolved in methyl iodide (60 mL) and stirred at room temperature for 3 days. Concentration in vacuo gave **3d** as an amorphous solid: NMR ($CDCl_3$) δ 1.42 (s, 9), 1.1–2.7 (m, 8), 3.1 (s, 3), 3.25 (s, 3) 3–3.3 (m, 2), 3.6 (m, 2), 3.71 (s, 3), 4.3–4.7 (m, 2), 5.10 (s, 2), 5.37 (m, 1), 6.03 (d, 1, J = 7 Hz), 7.37 (s, 5), 8.07 (d, 1, J = 7 Hz).

(*S*)-3-[(*tert*-Butoxycarbonyl)amino]-2-oxo-1-pyrrolidineacetic Acid (4a). Boc-Met-Gly methyl ester (5 g, 15.6 mmol) was converted to its methyl sulfonium iodide as described above. The total amount of this salt was dissolved in 312 mL of 1:1 DMF–CH_2Cl_2 under nitrogen and cooled to 0 °C. Sodium hydride (1.5 g of a 50% mineral oil suspension, 31.5 mmol) was added all at once, and the mixture was stirred at 0 °C for 2.5 h. Methyl acetate (104 mL) followed by water (24 mL) was added, and the resultant solution left overnight at room temperature. The solution was concentrated in vacuo to a small volume and partitioned between water (50 mL) and CH_2Cl_2 (50 mL). The phases were separated, and the aqueous phase at pH 8 was acidified to pH 4 with 0.5 M citric acid. Continuous extraction with CH_2Cl_2 followed by concentration in vacuo gave crystalline product: 2.06 g (51%); mp 171–172 °C dec. A second crop weighed 0.31 g (8%). Spectral data for **4a**: IR (Nujol) 3350, 1745, 1705, 1670, 1530, 1170 cm^{-1}; NMR (Me_2SO-d_6) δ 1.37 (s, 9), 1.7–2.5 (m, 2), 3.30 (m, 2), 3.77 (d, 1, J = 17 Hz), 4.04 (d, 1, J = 17 Hz), 4.0 (m, 1), 7.05 (br d, 1, J = 7.5 Hz), 12.87 (br m, 1); $[\alpha]^{24}_D$ −31.2° (*c* 1.0, MeOH).

Anal. Calcd for $C_{11}H_{18}N_2O_5$: C, 51.16; H, 7.02; N, 10.85. Found: C, 51.03; H, 7.34; N, 11.02.

(*S*)-3-[(*tert*-Butoxycarbonyl)amino]-2-oxo-1-pyrrolidine-(*S*)-4-methyl-2-pentanoic Acid (4b). From sulfonium salt **3b** (9.13 g, 17.7 mmol) was prepared, as described for **4a** above, **4b**: 2.2 g (40%); mp 154–155 °C; IR (Nujol) 3400, 1755, 1725, 1675, 1525, 1170 cm^{-1}; NMR (Me_2SO-d_6) δ 0.84 (d, 3, J = 7 Hz), 0.91 (d, 3, J = 7 Hz), 1.39 (s, 9), 1.5–1.9 (m, 4), 2.28 (m, 1), 3.26 (dd, 2, J_1 = 8 Hz, J_2 = 4 Hz), 4.12 (dd, 1, J_1 = 18 Hz, J_2 = 8 Hz), 4.56 (dd, 1, J_1 = 12 Hz, J_2 = 5 Hz), 7.20 (d, 1, J = 8 Hz), 13 (br s, 1); $[\alpha]^{24}_D$ +22.1° (*c* 1.0, MeOH).

Anal. Calcd for $C_{15}H_{26}N_2O_5$; C, 57.31; H, 8.34; N, 8.91. Found: C, 57.09; H, 8.60; N, 8.97.

(*S*)-3-[(*tert*-Butoxycarbonyl)amino]-2-oxo-1-pyrrolidine-(*S*)-3-phenyl-2-propionic Acid (4c). From sulfonium salt **3c** (7.81 g, 14.2 mmol) was prepared as described for **4a** above **4c** (2.5 g, 51%) as an amorphous solid. NMR analysis indicated the presence of 12–15% of a diastereomeric impurity from racemization at the phenylalanine α-carbon. Chromatography on silica gel (120:5:1:1 EPAW) furnished 1.46 g of amorphous **4c** and 82 mg of the diastereomer. Spectral data for **4c**: IR (CH_2Cl_2) 3400, 2950, 1710, 1500, 1160 cm^{-1}; NMR ($CDCl_3$) δ 1.43 (s, 9), 1.85 (m, 1), 2.48 (m, 1), 3.03 (dd, 1, J_1 = 14 Hz, J_2 = 12 Hz), 3.23 (dd, 1, J_1 = 16 Hz, J_2 = 10 Hz), 3.43 (m, 2), 4.01 (dd, 1, J_1 = 15 Hz, J_2 = 9 Hz), 5.08 (dd, 1, J_1 = 15 Hz, J_2 = 9 Hz), 5.29 (br s, 1), 7.24 (m, 5); $[\alpha]^{24}_D$ −63.5° (*c* 1.0, MeOH).

Anal. Calcd for $C_{18}H_{24}N_2O_5$: C, 62.07; H, 6.90; N, 8.04. Found: C, 61.71; H, 7.05; N, 7.80.

(*S*)-3-[(*tert*-Butoxycarbonyl)amino]-2-oxo-1-pyrrolidine-(*S*)-6-[(benzyloxycarbonyl)amino]-2-heptanoic Acid (4d). From sulfonium salt **3d** (10 g, 15.3 mmol) was prepared, as described for **4a** above, **4d** (4.6 g, 58%, corrected for 1 equiv of DMF). A modification of the workup in this case used glacial acetic acid to destroy excess NaH. A sample of **4d** crystallized on being allowed to stand, mp 137–139 °C. Recrystallization from EtOAc gave **4d**: mp 141.5–143 °C; IR ($CHCl_3$) 3400, 2950, 1710, 1505 cm^{-1}; NMR (CD_3OD) δ 1.42 (s, 9), 1.1–2.5 (m, 8), 3.08 (t, 2, J = 6 Hz), 3.2–3.5 (m, 2), 4.3 (br t, 1, J = 9 Hz), 4.54 (dd, 1, J_1 = 11 Hz, J_2 = 6 Hz), 5.03 (s, 2), 7.29 (s, 5); $[\alpha]^{24}_D$ −31.79 (*c* 0.77, MeOH).

Anal. Calcd for $C_{23}H_{33}N_3O_7 \cdot 0.5H_2O$: C, 58.46; H, 7.25; N, 8.89. Found: C, 58.43; H, 7.56; N, 8.94.

(*S*)-3-[(*tert*-Butoxycarbonyl)-(*R*)-alanylamino]-2-oxo-1-pyrrolidine-(*S*)-3-phenyl-2-propionic Acid. The sulfonium salt of Boc-D-Ala-Met-Phe-OMe was prepared as described for compounds **3a–d**. From this salt (1.93 g, 3.1 mmol) was prepared as described for **4a** above the expected lactam tripeptide acid (500 mg, 50%). A sample of purified material was obtained by chromatography on silica gel (solvent system D): IR (CH_2Cl_2) 3000, 1720, 1690, 1500, 1170 cm^{-1}; NMR ($CDCl_3$) δ 1.32 (d, 3, J = 6 Hz), 1.41 (s, 9), 1.89 (m, 1), 2.45 (m, 1), 3.03 (t, 1, J = 14 Hz), 3.25 (m, 1), 3.38 (t, 1, J = 9 Hz), 3.47 (dd, 1, J_1 = 8 Hz, J_2 = 5 Hz), 4.28 (m, 1), 5.03 (dd, 1, J_1 = 6 Hz, J_2 = 4 Hz), 5.51 (m, 1), 7.18–7.30 (m, 5), 7.43 (m, 1); $[\alpha]^{24}_D$ −25.7° (*c* 1, MeOH); TLC R_f 0.33 (system F).

N^{α}-(*tert*-Butoxycarbonyl)-N^{δ}-(carboxymethyl)ornithine (7a). N^{α}-Boc-N^{δ}-Cbz-Orn (11 g, 0.03 mol), glyoxylic acid hydrate (6.7 g), and 10% Pd on carbon catalyst were mixed in methanol (50 mL), water (35 mL), and acetic acid (5 mL). The mixture was hydrogenolyzed on a Parr apparatus until the monoalkylation product was major by TLC solvent system A (16 h), followed by filtration through Supercel. The catalyst was washed with 1:1 methanol–water, and the filtrate was concentrated in vacuo. Crystallization from methanol–ether gave 4.66 g (54%) of **7a**: mp 150 °C dec; IR (Nujol) 3300, 2670, 2630, 1730, 1690, 1550, 1510, 1390, 1225, 1170 cm^{-1}; NMR (Me_2SO-d_6) δ 1.37 (s, 9), 1.65 (m, 4), 2.82 (m, 2), 3.32 (m, 2), 3.83 (m, 1), 7.78 (br d, 1), 9.30 (br s, 3); $[\alpha]^{24}_D$ +2.59° (*c* 0.966, MeOH).

Anal. Calcd for $C_{18}H_{26}N_2O_6$: C, 49.65; H, 7.64; N, 9.65. Found: C, 49.54; H, 7.60; N, 9.99.

(*S*)-3-[(*tert*-Butoxycarbonyl)amino]-2-oxo-1-piperidineacetic Acid (8a). Diacid **7** (21.6 g, 74.5 mmol) was stirred in 700 mL of DMF with heating at 55 °C for 2 h. The solution was concentrated in vacuo to an oil which was crystallized from ethyl acetate–hexane to give three crops of product: 19.1 g (94%); mp 113–116 °C (first crop); IR (smear) 3300, 2950, 1720, 1555, 1495, 1360, 1070 cm^{-1}; NMR ($CDCl_3$) δ 1.44 (s, 9), 1.75 (m, 1), 1.99 (m, 2), 2.42 (m, 1), 3.36–3.56 (m, 2), 3.89 (d, 1, J = 15 Hz), 4.15 (m, 1), 4.34 (d, 1, J = 15 Hz), 5.62 (m, 1), 7.8 (br m, 1); $[\alpha]^{24}_D$ −22.14° (*c* 0.998, MeOH).

Anal. Calcd for $C_{12}H_{20}N_2O_5$: C, 52.93; H, 7.40; N, 10.29. Found: C, 52.99; H, 7.47; N 10.28.

(18) Prepared from 2% cross-linked chloromethylated polystyrene resin according to standard Merrifield technique. Cf.: Stewart, J. M.; Young, J. D., "Solid Phase Peptide Synthesis"; W. H. Freeman: San Francisco, CA, 1968.

(19) Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.

(*S*)-3-[(*tert*-Butoxycarbonyl)amino]-2-oxo-1-azepineacetic Acid (8b). N^{α}-Boc-N^{ϵ}-Cbz-Lys methyl ester[9] (5.9 g, 15 mmol) and glyoxylic acid hydrate (1.7 g) were dissolved in 40 mL of methanol and hydrogenated on a Parr apparatus in the presence of 10% Pd on carbon. When the monoalkylation product was major by TLC solvent system B (3 h), the mixture was filtered through Supercel and concentrated in vacuo to a foam. Preparative TLC (solvent system B) gave a sample of pure **7c** as an oil: IR (CH_2Cl_2) 3400, 2930, 1750, 1720, 1650, 1590, 1500, 1360, 1170, 1050, 1020 cm^{-1}; NMR ($CDCl_3$) δ 1.42 (s, 9), 1.4–2.0 (m, 6), 2.85 (m, 2), 3.45 (m, 2), 3.70 (s, 3), 4.20 (m, 1), 5.60 (m, 1), 9.02 (m, 3).

Crude **7c** (4.6 g, 14.5 mmol) and triethylamine (2.7 mL, 17.9 mmol) in 1650 mL of acetonitrile were heated at reflux for 3 days. The solution was cooled and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (100 mL) and washed with 0.5 M citric acid (3 × 30 mL) and 1 N aqueous $NaHCO_3$ (2 × 30 mL). The basic extracts were combined, acidified with 0.5 M citric acid, and extracted with CH_2Cl_2 (6 × 50 mL). The combined extracts were dried over Na_2SO_4, filtered, and concentrated in vacuo to a white foam (1.05 g, 25%). Spectral data for **8b**: IR (CH_2Cl_2) 3400, 2900, 1710, 1650, 1490, 1360, 1170, 1060, 1020, 975 cm^{-1}; NMR ($CDCl_3$) δ 1.44 (s, 9), 1.5–2.1 (m, 6), 3.21 (dd, 1, J_1 = 4 Hz, J_2 = 16 Hz), 3.5–3.9 (m, 1), 4.21 (s, 2), 4.48 (td, 1, J_1 = 1.5 Hz, J_2 = 5 Hz), 6.00 (d, 1, J = 6 Hz), 9.87 (s, 1); $[\alpha]^{24}_D$ −3.06° (*c* 0.98, MeOH).

Anal. Calcd for $C_{13}H_{22}N_2O_5$: C, 54.53; H, 7.74; N, 9.78. Found: C, 54.18; H, 7.96; N, 9.47.

S-(Acetamidomethyl)-N^{α}-phthalyl-(*R*)-cysteine (11). Compound 11 was prepared according to the procedure of Nefkens[11] from Acm-(*R*)-Cys (3.84 g, 20 mmol) and *N*-(ethoxycarbonyl)phthalimide (4.5 g, 21 mmol) with a reaction time of 90 min. The amorphous product was chromatographed on silica gel (500 g) by beginning with solvent system D and gradually increasing the polarity to solvent system E. The product (5.71 g, 90%) was obtained as a foam. Crystallization of a portion from 1:1 MeOH–H_2O gave the product: mp 186–188 °C; IR (Nujol) 3400, 1790, 1740, 1720, 1660, 1550, 1245 cm^{-1}; NMR (Me_2SO-d_6) δ 1.83 (s, 3), 3.0–3.5 (AB of ABC pattern, 2), 4.03 (dd, 1, J_1 = 13 Hz, J_2 = 6 Hz), 4.43 (dd, 1, J_1 = 13 Hz, J_2 = 6 Hz), 5.05–5.25 (C of ABC pattern, 1), 7.99 (s, 4), 8.53 (br t, 1, J = 6 Hz), 13 (br m, 1); $[\alpha]^{24}_D$ −120.3° (*c* 1.0, MeOH).

Anal. Calcd for $C_{14}H_{14}N_2O_5S$: C, 52.17; H, 4.38; N, 8.69. Found: C, 52.09; H, 4.52; N, 8.62.

S-(Acetamidomethyl)-N^{α}-phthalyl-(*R*)-cysteinylglycine Methyl Ester (12). The compound was prepared according to the procedure described for **2d** from *S*-(acetamidomethyl)-N^{α}-phthalyl-(*R*)-cysteine (18 g, 55.9 mmol) and glycine methyl ester hydrochloride (7.1 g, 56.6 mmol). After extractive workup with methylene chloride as the organic phase, concentration in vacuo gave a crystalline mass which was broken up and washed with methylene chloride and 1:1 methylene chloride–hexane: 16.9 g (77%); mp 152–160 °C. Recrystallization from CH_2Cl_2–MeOH–hexane gave a sample with a melting point of 160–161.5 °C: IR (Nujol) 1780, 1720, 1670 cm^{-1}; NMR (Me_2SO-d_6) δ 1.83 (s, 3), 3.30 (m, 2), 3.63 (s, 3), 3.84 (m, 2), 4.00 (dd, 1, J_1 = 13 Hz, J_2 = 6 Hz), 4.37 (dd, 1, J_1 = 13 Hz, J_2 = 6 Hz), 5.02 (dd, 1, J_1 = 11 Hz, J_2 = 5 Hz), 7.97 (s, 4), 8.4–8.8 (m, 2); $[\alpha]^{24}_D$ −34.84° (*c* 0.7, MeOH).

Anal. Calcd for $C_{17}H_{19}N_3O_6S$: C, 51.90; H, 4.87; N, 10.68. Found: C, 51.87; H, 5.07; N, 10.31.

Methyl 4-Oxo-5-phthalyl-1,3-thiazineacetate (13). *S*-(Acetamidomethyl)-N^{α}-phthalyl-(*R*)-cysteinyl-glycine methyl ester (16 g, 40.7 mmol), *p*-toluenesulfonic acid (1.5 g), and paraformaldehyde (5 g, 55.6 mmol) were heated to reflux (110–115 °C) under nitrogen in 150 mL of 1,1,2-trichloroethane using a Dean–Stark trap to separate water. After 2 and 4.5 h, additional paraformaldehyde (12 and 1.7 g, respectively) was added to complete the reaction. After 5 h, the reaction mixture was cooled and concentrated in vacuo to a small volume. The material was filtered through silica gel (57 g) with 400 mL of 99:1 $CHCl_3$–MeOH. Fractions of 150 and 250 mL were collected. From the first fraction was obtained by crystallization from ethyl acetate 4.63 g (34%) of product: mp 180–182 °C; IR (CH_2Cl_2) 1780, 1755, 1730, 1675, 1620, 1470, 1220, 1175, 1110 cm^{-1}; NMR ($CDCl_3$) δ 2.97 (ddd, 1, J_1 = 14 Hz, J_2 = 6 Hz, J_3 = 3 Hz), 3.78 (s over t, 4, J = 12.5 Hz), 4.18 and 4.27 (dd and s, 3, J = 12.5 Hz), 4.97 (d, 1, J = 12.5 Hz), 5.19 (dd, 1, J_1 = 12.5 Hz, J_2 = 6 Hz), 7.84 (m, 4); mass spectrum (70 eV), *m/e* 334 (M^+), 187 (base peak); $[\alpha]^{24}_D$ +2.1° (*c* 1.0, DMF).

Anal. Calcd for $C_{15}H_{14}N_2O_5S$: C, 53.89; H, 4.22; N, 8.38. Found: C, 53.95, H, 4.24; N, 8.30.

Additional product obtained by crystallizing further crops and chromatographing mother liquors (500 g silica gel, $CHCl_3$ eluant) and crystallizing amounted to 2.95 g (22%). An NMR study with Kiralshift E_7 shows the product to be approximately a 3:2 mixture of enantiomers on the basis of the relative heights of the methyl ester peaks.

4-Oxo-5-phthalyl-1,3-thiazineacetic acid (14). A solution of ester **13** (4.5 g, 13.5 mmol) in 210 mL of THF, 70 mL of water, and 35 mL of concentrated hydrochloric acid was refluxed under nitrogen for 4.25 h. The solution was cooled, diluted with 350 mL of water, and extracted with methylene chloride (4 × 200 mL). The combined extracts were dried over Na_2SO_4 and concentrated in vacuo. Crystallization from CH_2Cl_2/THF gave four crops of product: 3.65 g (85%); mp 225–227 °C; IR (Nujol) 1780, 1750, 1725, 1625, 1200, 1175, 1110 cm^{-1}; NMR (Me_2SO-d_6) δ 3.16 (ddd, 1, J_1 = 14 Hz, J_2 = 6 Hz, J_3 = 2.5 Hz), 3.57 (t, 1, J = 12 Hz), 3.99 and 4.21 (AB system, 2, J = 18 Hz), 4.46 and 4.76 (AB system, 2, J = 13 Hz, d at 4.46 split into dd, J_2 = 2.5 Hz), 4.92 (dd, 1, J_1 = 12 Hz, J_2 = 6 hz), 7.90 (s, 4); $[\alpha]^{24}_D$ −0.2° (*c* 1.0, DMF).

Anal. Calcd for $C_{14}H_{12}N_2O_5S$: C, 52.50; H, 3.78; N, 8.75. Found: C, 52.39; H, 3.78; N, 8.58.

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