Cycloserine Peptides[1]

REUBIN A. PAYNE AND CHARLES H. STAMMER[2]

Chemistry Department, University of Georgia, Athens, Georgia

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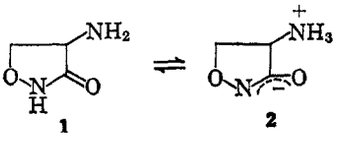
The synthesis of glycyl-DL-cycloserine, DD,LL-alanylcycloserine, and DL,LD-alanylcycloserine has been accomplished. A cycloserine derivative having the isoxazolidone ring blocked by a trityl group was prepared and shown to be a key intermediate in the synthesis of these compounds.

Our long-standing interest in the chemistry of cycloserine and its derivatives has led us to investigate the synthesis of cycloserine peptides. It has been established[3] that D-cycloserine (**1**) inhibits the incorporation of D-alanine into the cell wall peptide of certain bacteria by inhibition of L-alanine racemase and D-Ala-D-Ala synthetase enzymes. It was our intention to synthesize D-Ala-D-CS (CS = cycloserine) in order to determine its potential as an inhibitor of the synthetase enzyme and the results of these efforts are reported in this paper.

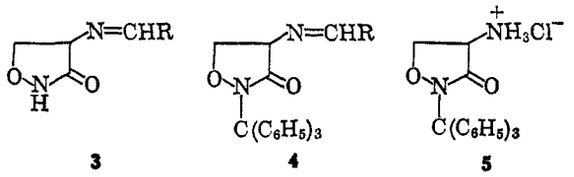
Several attempts to acylate cycloserine with appropriately derivatized amino acids gave complex mixtures which were not resolved. This result, apparently, is due to the fact that cycloserine exists to a large extent as the zwitterion,[4] **2**, making not only the



1 **2**

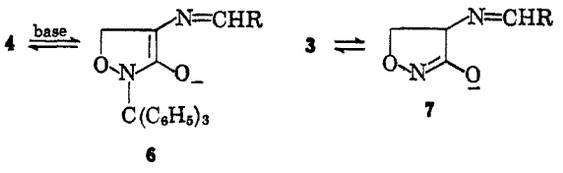
amino group, but also the centers of negative charge in the ambident cyclic hydroxamic acid anion available for acylation. Derivatization, then, at either of these two anionic sites should leave only the amino group available to an acylating agent. The sensitivity of the isoxazolidone ring to hydrogenolysis and to hydrolysis in acid media[5] precluded the use of many protecting groups commonly used in peptide synthesis. We found, however, that the ring was stable to anhydrous hydrogen bromide in acetic acid and to hot 50% aqueous acetic acid, reagents used to remove the trityl protecting group. The desired cycloserine derivative was, then, a ring-tritylated compound.

In some earlier work, we had prepared some 2-alkylated cycloserines; thus, a synthetic sequence, shown below, was already in hand for the preparation of a 2-trityl cycloserine. The Schiff base[6] **3** (R = 5-



3 **4** **5**

cyclosalicylidene) was tritylated rapidly in acetone with potassium carbonate as acid scavenger giving the trityl compound **4**. An infrared band at 1710 cm^{-1} in the spectrum of this product was consistent with the N-alkylated structure **4**, since O-alkylation would require the presence of an azomethine group (–N=C–) absorbing at lower frequency. Consistent with our earlier work[6] on the acetylation of **3**, was the finding that the 2-trityl compound **4** was completely racemized during the tritylation. Although thermal racemization of **3** was quite slow[6] and its racemization in the presence of bases was somewhat faster,[7] the 2-substituted Schiff bases racemized at rates comparable to their rates of formation. This is apparently due to rapid enolization of the carbonyl group giving the new enol anion **6** and destroying the steric integrity



6 **7**

of the asymmetric center at the 4 position.[8] Enolization of this type is kinetically unfavorable in **3** since the formation of enolate **7** is preferred; consequently, **3** is racemized much more slowy than **4**.

The racemic Schiff base **4** was then readily converted into the desired blocked cycloserine **5** by careful acid hydrolysis. The treatment of **5** with *ca.* 1 *N* HBr in glacial acetic acid gave, as shown by paper chromatography, cycloserine as the sole product.[9] With **5** in hand we had the blocked cycloserine necessary to the successful synthesis of the desired peptides.

We proceeded to couple **5**, using the mixed anhydride procedure,[10] with N-trityl-, N-*t*-butoxycarbonyl- and N-trifluoroacetylglycine. Each of the resulting blocked dipeptides was a crystalline, readily characterizable substance having acceptable elemental analyses[11] and spectra consistent with the proposed structures **8**. Both **8a** and **b** were smoothly converted into the

(1) This work was supported by a National Aeronautics and Space Administration Traineeship and is extracted from the thesis of R. A. P. which was submitted in partial fulfillment of the requirements for a Master of Science degree, University of Georgia, 1967.

(2) To whom inquiries should be addressed.

(3) (a) J. L. Strominger, R. H. Threnn, and S. S. Scott, *J. Amer. Chem. Soc.*, **81**, 3803 (1959); (b) E. Ito and J. L. Strominger, *J. Biol. Chem.*, **237**, 2696 (1962).

(4) J. B. Nielands, *Arch. Biochem. Biophys.*, **62**, 151 (1956).

(5) F. A. Kuehl, *et al.*, *J. Amer. Chem. Soc.*, **77**, 2344 (1955); P. H. Hidy, *et al.*, *ibid.*, 2345 (1955).

(6) C. H. Stammer and J. D. McKinney, *J. Org. Chem.*, **30**, 3436 (1965).

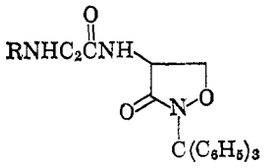
(7) Unpublished results.

(8) The asymmetric center in **4** is flanked by carbonyl and azomethine groups as is the asymmetric center of azlactones. M. Goodman and coworkers (Abstracts, 154th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1967, p 345) have shown that these compounds very rapidly racemize in the presence of bases.

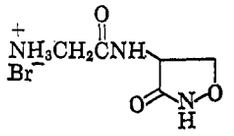
(9) If the HBr concentration was too high or the deblocking reaction was allowed to proceed too long, a second ninhydrin positive material was formed which had the same R_f as serine amide. Since it is difficult, mechanistically, to rationalize the formation of this product from cycloserine and HBr/HOAc, we are investigating this further.

(10) N. Izumiya and J. P. Greenstein, *Arch. Biochem. Biophys.*, **52**, 203 (1954).

(11) A great deal of difficulty was experienced in obtaining acceptable values on 2-trityl cycloserine derivatives. Since the completion of this work, we have found that purification of **5** through its free base reduces these problems.



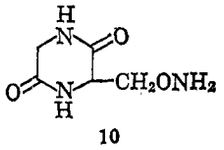
8a, R = *t*-BOC
b, R = $(C_6H_5)_3C$—
c, R = CF_3C(=O)—



9

crystalline dipeptide hydrobromide **9** with HBr–HOAc, as expected. Attempts to remove only the *t*-butoxycarbonyl (*t*-BOC) group from **8a** using hydrogen chloride in aprotic solvents failed to afford tractable products and the deblocking of **8c** in basic solution also failed. We had hoped to prepare the 2-trityl dipeptide by these means so that the peptide chain might be extended before detritylation. We did find that **9** itself could be benzoylated, albeit in low yield, using the standard Schotten–Baumann benzoylation conditions giving N-benzoylglycyl-DL-cycloserine. Possibly the peptide chain can be extended by direct acylation of **9** at high pH using the appropriate amino acid derivatives.

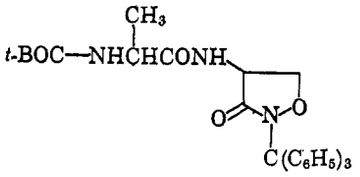
The dipeptide hydrobromide **9** rapidly cyclized at room temperature to the aminoxymethyl-2,5-piperazinedione (**10**) when neutralized with Amberlite IR-4B,



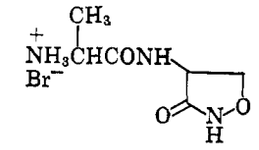
10

a weakly basic resin. This rearrangement was not altogether unexpected, since it is known that dipeptide esters[12] also slowly ring close to give piperazinediones.[13] The high rate at which **9** was converted[14] into **10** is another indication of the acylating power of the isoxazolidone ring. The piperazine derivative **10** was readily characterized by spectral and analytical data and the presence of the aminoxy function was shown by its conversion into a *p*-nitrobenzylidene derivative.

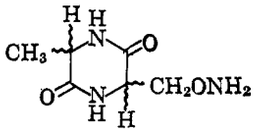
The required "protected" cycloserine (**5**) now in hand, we attacked the problem of alanylcycloserine synthesis. This was, of course, complicated by the fact that our blocked cycloserine was racemic and would afford diastereomeric mixtures on coupling with both active and racemic amino acid derivatives.[15] When **5** was coupled with *t*-BOC-DL-alanine, two racemic diastereomers were obtained, **11a** and **b**, each of which could be deblocked to give an alanylcycloserine salt, **12a** and **b**. Two 2,5-piperazinediones, **13a** and **b**, were formed when these dipeptide salts were neutralized. We tentatively assigned the *cis* configuration to **13a** and the *trans* configuration to **13b** based on an examination of Dreiding models and a comparison of melting points. We might expect the higher melting diastereomer (**13a**)



11a, mp 164°
b, mp 248°



12a, b



13a, mp >300°
b, mp 196°

to have the more symmetrical molecule. The large amount of double-bond character[16] in the amide C–N bond causes the 2,5-piperazinedione ring to exist in a quasi-boat conformation which places the 3 and 6 substituents in either quasi-axial or quasi-equatorial positions. In *cis* **13**, then, the 3,6 substituents should both reside in *quasi*-equatorial[17] conformations giving a more symmetrical structure than *trans* **13** which necessarily has one substituent quasi-equatorial and the other *quasi*-axial. Thus **13a**, the high melting isomer, should have the *cis* configuration.[18] Further work supported this assignment.

When *t*-BOC-D-alanine was coupled with 2-trityl-DL-cycloserine (**5**), a mixture of diastereomeric peptides was again obtained. These were not separable by fractional crystallization and the mixture was converted directly through deblocking and resin treatment into a mixture of piperazinediones. Recrystallization of this mixture afforded a product melting at about 300°, $[\alpha]_D$ +21.3°. The high melting point of this material indicates a *cis* configuration and the positive optical rotation supported this conclusion, since the dimer of D-cycloserine, *cis*-3,6-bisaminoxymethyl-2,5-piperazinedione, also has a positive rotation.[19] If these structural assignments are correct, then the blocked dipeptide **11a** must be the DD,LL racemate and **11b** is the DL,LD racemate.

The recent work of Halpern and coworkers[20] provided confirmation for these assignments. We found that the methyl resonances of **11a** and **b** occurred at 74 and 70 cps, respectively, indicating that **11a** was the DD,LL isomer since its methyl resonance was the more deshielded. Thus, the three pieces of evidence used to deduce the configurations of the alanyl dipeptides, **12a** (DD,LL) and **b** (DL,LD), are consistent and constitute

(12) J. P. Greenstein and M. Winitz, "Chemistry of Amino Acids," Vol. 2, John Wiley and Sons, Inc., New York, N. Y., 1961, p 796.

(13) The rapid formation of **10** from **9** at neutral pH also tends to support an earlier speculation[6] that an aminoxyalanylcycloserine derivative is intermediate in cycloserine dimer formation.

(14) The eluate from the resin column was ninhydrin negative almost immediately after collection.

(15) For biological testing purposes, however, we felt it advantageous to make all the possible isomers.

(16) R. C. Elderfield, "Heterocyclic Compounds," Vol. 6, John Wiley and Sons, Inc., New York, N.Y., 1957, p 440.

(17) The models show large "flagpole–flagpole" interactions when the substituents are quasi-axial.

(18) A recent report by K. D. Kopple and D. H. Marr, *J. Amer. Chem. Soc.*, **89**, 6193 (1967), indicates that in 3-benzyl-2,5-piperazinediones the piperazine ring is flat. This may be due to interactions peculiar only to this system and does not invalidate our arguments invoking the symmetry of the *cis* and *trans* isomers, **13a** and **b**.

(19) H. Brockmann and H. Musso [*Chem. Ber.*, **89**, 241 (1965)] reported a positive rotation for *cis*-DD-3,6-dimethyl-2,5-piperazinedione (from D-alanine) and a melting point (288–290°) greater than that of the *trans* compound (277–278°).

(20) B. Halpern, L. F. Chew, and B. Weinstein, *J. Amer. Chem. Soc.*, **89**, 5051 (1967); B. Halpern, D. E. Nitecki, and B. Weinstein, *Tetrahedron Lett.*, 3075 (1967). In some ten dipeptides, these workers found the DD,LL isomers to have the alanine methyl resonance some 5–10 cps downfield of the DL,LD compound.

a strong case for our assignments. We were unable to characterize **12a** and **b**, other than through their conversions into piperazinediones **13a** and **b** since the dipeptides were amorphous highly hydroscopic solids.

Experimental Section

N-(5-Chlorosalicylidene)-D-cycloserine,[6] N-triphenylmethylglycine,[21] N-*t*-butoxycarbonylglycine,[22] N-*t*-butoxycarbonyl-DL-alanine,[22] N-*t*-butoxycarbonyl-D-alanine,[21] and N-trifluoroacetylglycine[23] were prepared according to known methods. All melting points were taken on a Nalge hot stage and are corrected. Infrared spectra were determined either on a Perkin-Elmer Model 137 or Model 237B spectrometer; ultraviolet spectra were determined on a Perkin-Elmer Model 202 ultraviolet-visible spectrometer; and nmr spectra were determined on a Varian Associates Model A-60 nmr spectrometer. All microanalyses were performed by Midwest Microlab, Inc., Indianapolis, Ind.

N-(5-Chlorosalicylidene)-2-triphenylmethyl-DL-cycloserine (4).—To a solution of 4.82 g (20 mmol) of N-(5-chlorosalicylidene)-D-cycloserine in 100 ml of purified acetone[24] in a 250-ml, round-bottomed flask stirred magnetically and protected with a Drierite tube was added 3.04 g (22 mmol) of anhydrous powdered potassium carbonate. The suspension was stirred at room temperature for 5 min, 6.14 g (22 mmol) of triphenylmethyl chloride was added, and stirring was continued at room temperature for 2.5 hr. The resulting suspension was centrifuged and the supernatant liquid was concentrated to an oil *in vacuo*. The residual oil was dissolved in *ca.* 50 ml of anhydrous ether from which crystals immediately began to form. Filtration gave 7.43 g of yellow prisms, mp 155–167°. The filtrate afforded a second crop weighing 0.75 g (87% total yield). An analytical sample was prepared by recrystallization from acetone–methanol: mp 166–167°; uv ($CHCl_3$), 246 mμ (ε 15,090), 256 (13,140), and 339 (4702); ir (Nujol), 1710 (C=O), 1625 (C=N), 825 (C–Cl), 750–650 cm^{-1} (aromatic); nmr ($CDCl_3$), δ 4.4 (m, 3, cycloserine ring), 6.9 (d, 3), 7.3 (m, 15, $(C_6H_5)_3C$–), and 8.22 ppm (s, 1, –CH=). *Anal.* Calcd for $C_{29}H_{23}N_2O_3Cl$: C, 72.21; H, 4.77; N, 5.81; Cl, 7.31. Found: C, 71.89; H, 4.72; N, 6.10; Cl, 7.52.

2-Triphenylmethyl-DL-cycloserine Hydrochloride (5).—To a solution of 4.71 g (9.78 mmol) of N-(5-chlorosalicylidene)-2-triphenylmethyl-DL-cycloserine in 200 ml of dry DME[25] stirred magnetically was added dropwise 0.89 ml (11 mmol) of concentrated hydrochloric acid. The solution was stirred at ambient temperature for 30 min while the color changed from deep to light yellow and was evaporated to dryness *in vacuo* giving a yellow amorphous solid which was subsequently stirred magnetically in 100 ml of dry ether for 3 hr. Filtration gave 3.48 g (94% yield) of white amorphous solid.

An analytical sample was necessarily prepared by adding 0.38 g (1.0 mmol) of the product to 20 ml of 1% aqueous sodium bicarbonate and extracting the mixture with three 20-ml portions of methylene chloride. The combined organic layers were washed with 10 ml of saturated aqueous sodium chloride, dried over anhydrous magnesium sulfate, and filtered, and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in 50 ml of dry ether. The solution was cooled in an ice bath and anhydrous hydrogen chloride was passed over the surface of the cold solution causing a white precipitate to form immediately. The suspension was stirred for 5 min and filtered giving 0.22 g (58% yield) of white amorphous solid. A sample was recrystallized from methanol–ether for analysis: mp 149–152°, uv (CH_3OH), 210 mμ (ε 27,280), 237 (9120), and 265 (3294); ir (Nujol), 1700 (C=O), and 800–650 cm^{-1} (aromatic); nmr (acetone-d_6–D_2O), δ 5.5 (m, 3, cycloserine ring) and 7.3 ppm (m, 15, $(C_6H_5)_3C$–). *Anal.* Calcd for $C_{22}H_{21}N_2O_2Cl$: C, 69.37; H, 5.56; N, 7.36; Cl, 9.31. Found: C, 69.09; H, 5.84; N, 7.21; Cl, 9.07.

An acceptable analysis could not be obtained by several recrystallizations from methanol–ether of the crude hydrolysis product.

N-*t*-Butoxycarbonylglycyl-2-triphenylmethyl-DL-cycloserine (8a).—To a solution of 1.05 g (6.0 mmol) of *t*-butoxycarbonylglycine in 150 ml of dry ethyl acetate in a 250-ml, round-bottomed flask protected with a "Drierite" tube, stirred magnetically and held at −10°, was added 0.93 ml (6.6 mmol) of triethylamine and 0.87 ml (6.6 mmol) of isobutyl chloroformate. A white precipitate formed immediately and the suspension was stirred at −10° for 30 min. To this suspension was added 2.28 g (6.0 mmol) of 2-triphenylmethyl-DL-cycloserine hydrochloride and 0.87 ml (6.2 mmol) of triethylamine, the ice bath was removed, and the suspension was stirred at room temperature for 18 hr. The precipitated triethylamine hydrochloride (1.74 g) was removed by filtration. The filtrate was concentrated to an oil *in vacuo* which was dissolved in *ca.* 25 ml of dry ether. The white crystals which formed were collected by filtration (2.90 g, 96% yield), mp 204–206°. An analytical sample was prepared by recrystallization from DME–petroleum ether (bp 30–60°): mp 201–203°; ir (Nujol), 1715 (C=O, ring), 1695 (C=O, urethan), 1670 (C=O, amide I), 1550 (amide II), and 775–675 cm^{-1} (aromatic); nmr (acetone-d_6), δ 1.4 (s, 9, $(CH_3)_3C$–), 3.7–4.5 (m, 5, cycloserine ring and $-CH_2-$) and 7.3 ppm (m, 15, $(C_6H_5)_3C$–). *Anal.* Calcd for $C_{29}H_{31}N_3O_5$: C, 69.44; H, 6.23; N, 8.38. Found: C, 69.11; H, 6.40; N, 8.43.

N-Triphenylmethylglycyl-2-triphenylmethyl-DL-cycloserine (8b).—A solution of 2-triphenylmethyl-DL-cycloserine free base in dry[26] ethyl acetate was prepared by adding 0.38 g (1.0 mmol) of 2-triphenylmethyl-DL-cycloserine hydrochloride to 20 ml of 1% aqueous sodium bicarbonate and extracting the mixture with four 20-ml portions of methylene chloride. The combined organic layers were dried over anhydrous magnesium sulfate and filtered, and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in 20 ml of dry ethyl acetate and the solution was filtered before use. Simultaneously with the above procedure, 0.32 g (1.0 mmol) of N-triphenylmethylglycine was dissolved in 25 ml of dry ethyl acetate in a 50-ml round-bottomed flask protected from moisture with a Drierite tube and stirred magnetically. This solution was cooled to −10° and 0.154 ml (1.1 mmol) of triethylamine and 0.145 ml (1.1 mmol) of isobutyl chloroformate was added. A precipitate formed immediately and the mixture was stirred for 20 min at −10°. The precipitated triethylamine hydrochloride was removed by filtration and washed with 15 ml of dry ethyl acetate. To the combined filtrate and washing in a 100-ml, round-bottomed flask protected with a Drierite tube and magnetically stirred was added the above solution of 2-triphenylmethyl-DL-cycloserine and the resulting solution was stirred for 20 hr at ambient temperature during which time the product crystallized. Filtration afforded 0.43 g (67% yield) of white crystals, mp 250–253°. A second crop weighed 0.05 g (75% yield). An analytical sample was prepared by recrystallization from chloroform–propanol: mp 255–256°; ir (Nujol), 1720 (C=O, ring), 1660 (C=O, amide I), 1520 (amide II), and 800–675 cm^{-1} (aromatic); nmr ($CDCl_3$), δ 3.0 (s, 2, $-CH_2-$), 4.6 (m, 3, cycloserine ring), and 7.3 ppm (m, 30, $(C_6H_5)_3C$–). *Anal.* Calcd for $C_{43}H_{37}N_3O_3$: C, 80.22; H, 5.79; N, 6.53. Found: C, 78.30; H, 5.73; N, 6.80.

N-Trifluoroacetylglycyl-2-triphenylmethyl-DL-cycloserine (8c).—A solution of 2-triphenylmethyl-DL-cycloserine in dry methylene chloride was prepared by adding 0.38 g (1.0 mmol) of 2-triphenylmethyl-DL-cycloserine hydrochloride to 20 ml of 1% aqueous sodium bicarbonate and extracting the mixture with four 20-ml portions of methylene chloride. The combined organic layers were dried over anhydrous magnesium sulfate and filtered, and the filtrate was evaporated to dryness *in vacuo*. The residue was dissolved in 20 ml of dry methylene chloride. To this solution in a 50-ml, round-bottomed flask protected with a Drierite tube and stirred magnetically was added 0.17 g (1.0 mmol) of trifluoroacetylglycine and 0.19 g (0.92 mmol) of N,N′-dicyclohexylcarbodiimide. All the solid dissolved immediately and after 2 min a precipitate formed. Stirring was continued at room temperature for 4 hr and the solid, weighing 0.13 g was removed by filtration. The filtrate was evaporated *in vacuo* and 15 ml of ether was added to the residue. An insoluble solid (0.05 g) was removed by filtration and 10 ml of petroleum ether (bp 30–60°) was added to the filtrate causing the formation of 0.38 g (83%) of white crystals, mp 126–131°. An analytical sample

(21) L. Zervas and D. M. Theodoropoulas, *J. Amer. Chem. Soc.*, **78**, 1359 (1956).

(22) R. Schwyzer, P. Sieber, and H. Koppeler, *Helv. Chim. Acta*, **42**, 2622 (1959).

(23) F. Weygand and R. Geiger, *Chem. Ber.*, **89**, 647 (1956).

(24) The acetone was purified by refluxing reagent grade acetone over potassium permanganate for 24 hr, distilling it from the potassium permanganate, and drying it over anhydrous potassium carbonate.

(25) DME = 1,2-dimethoxyethane. The DME was dried by refluxing it over and distilling it from sodium.

(26) The ethyl acetate was dried over molecular sieves (Linde Type 4A).

was prepared by recrystallization from benzene–petroleum ether: mp 153–156°; ir (Nujol), 1725 (C=O, ring) 1685 (C=O, acetyl), 1655 (C=O, amide I) 1555 (amide II), 1180 (C–F), and 750–675 cm^{-1} (aromatic); nmr (acetone-d_6), δ 3.0 (s, 2, $-CH_2-$), 4.2 (m, 3, cycloserine ring), and 7.3 ppm (m, 18, $(C_6H_5)_3C-$ and benzene). *Anal.* Calcd for $C_{26}H_{22}N_3O_4F_3 \cdot {}^1/_2C_6H_6$: C, 64.92; H, 4.70; N, 7.83. Found: C, 64.70; H, 5.20; N, 7.93.

The elemental analysis of this compound was a function of the temperature and duration of drying *in vacuo*. Variable amounts of benzene (see nmr data) were apparently present in the analytical samples. The analysis reported here is the most acceptable one obtained.

N-Glycyl-DL-cycloserine Hydrobromide (9). A.—To a stirred suspension of 4.00 g (6.22 mmol) of N-triphenylmethylglycyl-2-triphenylmethyl-DL-cycloserine in 20 ml of glacial acetic acid was added 20 ml of 1 *N* hydrogen bromide[27] in glacial acetic acid. All the solid dissolved within 3 min followed by the immediate precipitation of a granular solid. The suspension was stirred at room temperature for 5 min more and was poured slowly into 300 ml of dry ether which was magnetically stirred. The white suspension was stirred for 30 min at room temperature and filtered, affording 1.50 g (100% yield) of a white, amorphous, hygroscopic solid. Further purification was obtained by dissolving the solid in 30 ml of dry ethanol and slowly adding 75 ml of dry ether, causing the precipitation of 1.25 g of amorphous solid; the ir spectrum (Nujol) showed bands at 1725 (C=O, ring), 1690 (C=O, amide I), and 1555 cm^{-1} (amide II).

B.—To a stirred suspension of 2.00 g (4.0 mmol) of N-*t*-butoxycarbonylglycyl-2-triphenylmethyl-DL-cycloserine in 20 ml of glacial acetic acid in a 50-ml erlenmeyer flask was added 13 ml of 1 *N* hydrogen bromide in glacial acetic acid. All the solid did not dissolve before a granular solid precipitated. The suspension was stirred at room temperature for 5 min and was poured slowly into 300 ml of dry ether magnetically stirred. The white suspension was stirred for 1 hr at room temperature and filtered, affording 1.04 g (108% yield) of slightly pink, amorphous, hygroscopic solid. The infrared spectrum of a sample obtained by dissolving the crude product in ethanol followed by addition of ether was identical with that of the sample from procedure A.

N-Benzoylglycyl-DL-cycloserine.—To 3.4 ml (3.3 mmol) of 0.965 *N* aqueous sodium hydroxide in a round-bottomed flask was added 240 mg (1 mmol) of N-glycyl-DL-cycloserine hydrobromide. To this solution was added 0.13 ml (1.1 mmol) of benzoyl chloride. The flask was stoppered and shaken vigorously for 5 min, during which time heat was evolved. The solution was diluted with 7 ml of water, extracted with two 15-ml portions of ethyl acetate, and diluted with 10 ml of ethanol. After 1 hr, the solvent was removed *in vacuo*, the residue as dissolved in 10 ml of water, acidified to pH 4.5 with acetic acid, and extracted with two 10-ml portions of ethyl acetate. The combined organic layers were dried over anhydrous magnesium sulfate and filtered, and the solvent was evaporated *in vacuo* giving 0.11 g (42% yield) of a white solid, mp 173–177°. An analytical sample was prepared by several recrystallizations from ethanol: mp 196–198°; ir (Nujol), 1695 (C=O, ring), 1650 (C=O, amide I, peptide), 1635 (C=O, amide I, aromatic), and 1530 cm^{-1} (amide II). *Anal.* Calcd for $C_{12}H_{13}N_3O_4$: C, 54.75; H, 4.98; N, 15.96. Found: C, 54.34; H, 5.26; N, 15.58.

DL-3-Aminoxymethyl-2,5-piperazinedione (10).—A solution of 0.32 g (1.3 mmol) of N-glycyl-DL-cycloserine hydrobromide in 75 ml of water was passed through a neutral column of 15 ml (37.5 mequiv) of Amberlite IR-4B. The column was washed with 75 ml of water and the combined effluents were lyophilized, giving 0.14 g (90% yield) of white powder. An analytical sample was prepared by recrystallization from water–ethanol: mp 300°; ir (Nujol), 1670 (C=O), 1335 (C–O), and 1010 cm^{-1} (N–O). *Anal.* Calcd for $C_5H_9N_3O_3$: C, 37.74; H, 5.70; N, 26.40. Found: C, 37.53; H, 5.94; N, 26.10.

DL-3-[N-(4-Nitrobenzylidene)aminoxymethyl]-2,5-piperazinedione.—A suspension of 97.7 mg (0.614 mmol) of 3-DL-aminoxymethyl-2,5-piperazinedione and 93.0 mg (0.615 mmol) of 4-nitrobenzaldehyde in 5 ml of dry methanol was stirred magnetically for 1 hr at room temperature. The solvent was evaporated *in vacuo*, the residue was dissolved in 6 ml of hot DMF, the solution was centrifuged, and 4 ml of water was added to the supernatant liquid. The white crystals which formed were recrystallized several times from DMF–water: mp 243–244°; ir (Nujol), 1675 (C=O), 1520 (C=N), 1350 (C–O), and 1020 cm^{-1} (N–O).

N-*t*-Butoxycarbonyl-DL-alanyl-2-triphenylmethyl-DL-cycloserine (11a,b).—To a solution of 2.84 g (15 mmol) of *t*-butoxycarbonyl-DL-alanine in 250 ml of dry ethyl acetate in a 500-ml, round-bottomed flask protected from moisture with a Drierite tube, stirred magnetically and held at −10°, was added 2.35 ml (16.5 mmol) of triethylamine and 2.20 ml (16.5 mmol) of isobutyl chloroformate. A white precipitate formed immediately and the suspension was stirred at −10° for 25 min. To this suspension was added 2.35 ml (16.5 mmol) of triethylamine and 5.71 g (15 mmol) of 2-triphenylmethyl-DL-cycloserine hydrochloride, the ice bath was removed, and the suspension was stirred at room temperature for 18.5 hr. The precipitated solid (6.46 g) was removed by filtration and the filtrate was concentrated to an oil *in vacuo*. The residue was dissolved in 25 ml of ether from which 4.60 g (60% of theory) of white crystals formed, mp 164–166°. An analytical sample was prepared by repeated recrystallization from diethylene glycol–dimethyl ether–petroleum ether: mp 191–193°; ir (Nujol), 1755 (C=O, ring), 1680 (C=O, urethan), 1665 (C=O, amide I), 1525 (amide II), and 775–700 cm^{-1} (aromatic); nmr ($CDCl_3$), δ 1.2 (d, 3, $-CH_3$), 1.4 (s, 9, $-CH_3$), 3.0 (m, 1, $-CH=$), 4.5 (m, 3, cycloserine ring), 5.3 (d, 1, urethan NH), 6.85 (broad singlet, 1, amide NH), 7.3 ppm (m, 15, $(C_6H_5)_3C-$). This racemate has been assigned the structure of DD,LL-N-*t*-butoxycarbonylalanyl-2-triphenylmethylcycloserine (**11a**). *Anal.* Calcd for $C_{30}H_{33}N_3O_5$: C, 69.88; H, 6.45; N, 8.15. Found: C, 68.86; H, 6.77; N, 8.07.

The solid filtered from the reaction mixture was washed with four 50-ml portions of water to remove the triethylamine hydrochloride and dried giving 2.78 g (36% of theory) of a white solid, mp 240–242°. An analytical sample was prepared by repeated recrystallization from DMF–ethanol–water (10:10:2): mp 247–248°; ir (Nujol), 1710 (C=O, ring), 1685 (C=O, urethan), 1665 (C=O, amide I), 1535 (amide II), and 705 cm^{-1} (aromatic); nmr (dimethylsulfoxide-d_6), δ 1.2 (d, 3 $-CH_3$), 1.4 (s, 9, $(CH_3)_3C-$), 4.2 (m, 3, cycloserine ring), 7.3 ppm (s, 15 H, $(C_6H_5)_3C-$). This racemate has been assigned the structure of DL,LD-N-*t*-butoxycarbonylalanyl-2-triphenylmethylcycloserine (**11b**). *Anal.* Calcd for $C_{30}H_{33}N_3O_5$: C, 69.88; H, 6.45; N, 8.15. Found: C, 69.13; H, 6.58; N, 8.51.

***rac-cis*-3-Aminoxymethyl-6-methyl-2,5-piperazinedione (13a).**—To a suspension of 2.06 g (4.0 mmol) of DD,LL-*t*-butoxycarbonylalanyl-2-triphenylmethylcycloserine (**11a**) in 20 ml of glacial acetic acid stirred magnetically was added 15 ml of 1 *N* hydrogen bromide in glacial acetic acid. The suspension was stirred at room temperature for 5 min and poured slowly into 300 ml of dry ether magnetically stirred. The white suspension was stirred for 1 hr at room temperature and filtered, affording 0.58 g (57% yield) of slightly pink, amorphous, hygroscopic solid. The solid was dissolved in 100 ml of water and passed through a column of 16 ml (40 mequiv) of Amberlite IR-4B. The column was washed with 100 ml of water and the combined effluents were lyophilized giving 0.32 g (81% yield) of a white amorphous solid. An analytical sample was prepared by recrystallization from methanol: mp >300°; ir (Nujol), 1690 (C=O), 1340 (C–O), 1010 cm^{-1} (N–O). *Anal.* Calcd for $C_6H_{11}N_3O_3$: C, 41.62; H, 6.40; N, 24.26. Found: C, 41.90; H, 6.69; N, 24.13.

***rac-cis*-3-[N-(4-Nitrobenzylidene)aminoxymethyl]-6-methyl-2,5-piperazinedione.**—A suspension of 17.3 mg (0.10 mmol) of *rac-cis*-3-aminoxymethyl-6-methyl-2,5-piperazinedione (**13a**) and 15.2 mg (0.10 mmol) of 4-nitrobenzaldehyde in 0.2 ml of water and 5 ml of methanol was stirred magnetically for 1 hr at room temperature. The solvent was evaporated *in vacuo*, the residue was dissolved in 3 ml of hot DMF and centrifuged, and 10 ml of water was added to the supernatant liquid. The white crystals which formed were recrystallized from DMF–water and washed with ethanol: mp 220–222°; ir (Nujol), 1685 (C=O), 1515 (C=N), 1340 (C–O), and 1005 cm^{-1} (N–O). *Anal.* Calcd for $C_{13}H_{14}N_4O_5$: C, 50.98; H, 4.61; N, 18.29. Found: C, 50.45; H, 5.15; N, 18.35.

***rac-trans*-3-Aminoxymethyl-6-methyl-2,5-piperazinedione (13b).**—To a suspension of 1.65 g (3.2 mmol) of DL,LD-N-*t*-butoxycarbonylalanyl-2-triphenylmethylcycloserine (**11b**) in 15 ml of glacial acetic acid stirred magnetically was added 12 ml of 1 *N*

(27) The 1 *N* hydrogen bromide in acetic acid was prepared by diluting 60 ml of 30–32% hydrogen bromide in acetic acid (obtained from Eastern Organic Chemicals, Co.) to 210 ml with glacial acetic acid.

hydrogen bromide in glacial acetic acid. The suspension was stirred at room temperature for 4 min and poured slowly into 225 ml of dry ether which was stirred magnetically. The white suspension was stirred for 1.5 hr at room temperature and filtered, affording 0.55 g (68% yield) of white, amorphous, hygroscopic solid. The solid was dissolved in 100 ml of water and passed through a column of 12 ml (30 mequiv) of Amberlite IR-4B. The column was washed with 100 ml of water and the combined effluents were lyophilized, giving 0.40 g (106% yield) of a white amorphous solid which exhibited a negative halogen test with silver nitrate. An analytical sample was prepared by recrystallization from methanol: mp 195–196°; ir (Nujol), 1690 (C=O), 1330 (C–O), and 1080 cm^{-1} (N–O); nmr (dimethylsulfoxide-d_6), δ 1.3 (d, 3, $-CH_3$), 3.7 (broad multiplet), and 8.1 ppm (broad doublet, –NH). *Anal.* Calcd for $C_6H_{11}N_3O_3$: C, 41.62; H, 6.40; N, 24.26. Found: C, 41.86; H, 6.63; N, 23.98.

rac-trans-**3-[N-(4-Nitrobenzylidene)aminoxymethyl]-6-methyl-2,5-piperazinedione.**—A suspension of 17.5 mg (0.10 mmol) of *rac-trans*-3-aminoxymethyl-6-methyl-2,5-piperazinedione and 15.2 mg (0.10 mmol) of 4-nitrobenzaldehyde in 0.2 ml of water and 5 ml of methanol was stirred magnetically for 1.5 hr at room temperature. The solvent was evaporated *in vacuo*, the residue was dissolved in 2 ml of hot DMF and centrifuged, and water was added to the supernatant liquid until it was slightly turbid. The crystals which formed were recrystallized from DMF–water: mp 244–245°; ir (Nujol), 1680 (C=O), 1665, (C=O, hydrogen bonded), 1335 (C–O), and 970 cm^{-1} (N–O). *Anal.* Calcd for $C_{13}H_{14}N_4O_5$: C, 50.98; H, 4.61; N, 18.29. Found: C, 50.70; H, 4.80; N, 18.24.

N-*t*-Butoxycarbonyl-D-alanyl-2-triphenylmethyl-DL-cycloserine.—To a solution of 2.84 g (15 mmol) of *t*-butoxycarbonyl-D-alanine in 250 ml of dry ethyl acetate in a 500-ml, round-bottomed flask protected from moisture with a Drierite tube, stirred magnetically and held at −10°, was added 2.35 ml (16.5 mmol) of triethylamine and 2.20 ml (16.5 mmol) of isobutyl chloroformate. A white precipitate formed immediately and the suspension was stirred at −5° for 25 min. To this suspension was added 2.35 ml (16.5 mmol) of triethylamine and 5.71 g (15 mmol) of 2-triphenylmethyl-DL-cycloserine hydrochloride, the ice bath was removed, and the suspension was stirred at room temperature for 16.5 hr. The solid, weighing 4.24 g, was removed by filtration, the filtrate was evaporated *in vacuo*, and the residue was dissolved in 25 ml of ether. No crystals formed and the solution was filtered and concentrated to an oil under a stream of anhydrous nitrogen, and the residue was covered with 60 ml of petroleum ether (bp 30–60°). Upon cooling and scratching, the oil solidified giving 7.13 g (92% yield) of a white amorphous solid, $[\alpha]^{27}D$ +9.4° (*c* 2.04, $CHCl_3$). Crystallization attempts from standard solvent systems were unsuccessful.

Optically Active Mixture of DD-*cis*- and LD-*trans*-3-Aminoxymethyl-6-methyl-2,5-piperazinediones.—To a solution of 5.15 g (10 mmol) of crude N-*t*-butoxycarbonyl-D-alanyl-2-triphenylmethyl-DL-cycloserine in 50 ml of glacial acetic acid stirred magnetically was added 35 ml of 1 *N* hydrogen bromide in glacial acetic acid. The solution was stirred for 5 min at room temperature, during which time a precipitate formed. This suspension was poured into 750 ml of dry ether magnetically stirred and the white suspension was stirred for 1 hr at room temperature and filtered affording 1.75 g (69%) of amorphous, hygroscopic solid. The solid (1.5 g) was dissolved in 200 ml of water and passed through a column of 45 ml (112 mequiv) of Amberlite IR-4B. The column was washed with 100 ml of water, and the combined effluents were lyophilized, giving 0.90 g (80% yield) of a slightly yellow amorphous solid. A sample of 0.80 g was dissolved in 30 ml of hot methanol from which 0.44 g (55% recovery) of white crystals formed: mp >300°; $[\alpha]^{28}D$ +19.6° (*c* 1.42, water). A second crop gave 0.08 g of white crystals, $[\alpha]^{28}D$ +19.5° (*c* 1.53, water). The 0.44-g sample was recrystallized from 2.5 ml of water giving 0.30 g of white crystals which were recrystallized from 3 ml of water giving 0.12 g of white crystals, mp >300°, $[\alpha]^{29}D$ +21.3° (*c* 1.7, water); ir (Nujol), 1670 (C=O), 1340 (C–O), and 1000 cm^{-1} (N–O).

Registry No.—**4,** 16561-89-0; **5,** 16561-90-3; **8a,** 16561-91-4; **8b,** 16561-92-5; **8c,** 16561-93-6; **9,** 16561-94-7; **10,** 16561-95-8; **11a,** 16561-96-9; **11b,** 16561-97-0; **13a,** 16561-98-1; **13b,** 16561-99-2; N-benzoylglycyl-DL-cycloserine, 16562-00-8; DL-3-[N-(4-nitrobenzylidene)-aminoxymethyl]-2,5-piperazinedione, 16562-01-9; *rac-cis*-3-[N-(4-nitrobenzylidene)aminoxymethyl]-6-methyl-2,5-piperazinedione, 16562-02-0; *rac-trans*-3-[N-(4-nitrobenzylidene)aminoxymethyl]-6-methyl-2,5-piperazinedione, 16562-05-3; DD-*cis*-3-aminoxymethyl-6-methyl-2,5-piperazinedione, 16562-03-1; LD-*trans*-3-aminoxymethyl-6-methyl-2,5-piperazinedione, 16562-04-2.

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