Racemization Mechanism of Cysteine Dipeptide Active Ester Derivatives[1]

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The racemization rate constants of Z-Gly-L-Cys(Bzl)-OH and Z-L-Cys(Bzl)-OH active esters were practically identical, even though glycyl dipeptide active esters usually racemize 50–100 times faster than the amino acid active esters. This observation led to the conclusion that Z-Gly-L-Cys(Bzl)-ONp and other active esters do not racemize through the 5(4*H*)-oxazolone but through an enolization mechanism. The deuterium isotope effect was used to demonstrate that the removal of the α-hydrogen in the racemization is indeed the rate-determining step. Ac-Cys(Bzl)-OH was converted with DCC to the 5(4*H*)-oxazolone and deuterated with AcO^2H: during this procedure Ac_2O and Ac-(α-^{2}H)-DL-Cys(Bzl)-OH were formed, the latter was resolved with acylase I in the presence of Co^{2+} ions. H(α-^{2}H)-L-Cys(Bzl)-OH (II) was converted through Z-(α-^{2}H)-L-Cys(Bzl)-OH (V), Z-(α-^{2}H)-L-Cys(Bzl)-ONp (VI), and HBr·H-(α-^{2}H)-L-Cys(Bzl)-ONp (VII) to Z-Gly-(α-^{2}H)-L-Cys(Bzl)-ONp (VIII). The racemization rate constant k_r^{2H} for deuterated VIII in THF with Et_3N was 335×10^{-6} M^{-1} s^{-1}, while the k_r^H for the undeuterated VIII was 704×10^{-6} M^{-1} s^{-1}, indicating an isotope effect of 2.1. The 5(4*H*)- and 5(4^2*H*)-oxazolones, XII and XIII, were prepared from Z-Gly-L-Cys(Bzl)-OH and Z-Gly-(α-^{2}H)-L-Cys(Bzl)-OH with DCC under controlled conditions in 73% optical purity. Racemization of XII and XIII with Et_3N in THF is instantaneous while the coupling rate with H-Val-OMe is $k_c = 8.5 \times 10^{-2}$ M^{-1} s^{-1}; that is, $k_r \gg k_c$. The racemization rate of 5(4*H*)-oxazolone XII during coupling with H-Val-OMe is 0.34 M^{-1} s^{-1}, while the autoracemization rate of XII is 0.08 M^{-1} s^{-1}. These results support the conclusions that Z-Gly-Cys(Bzl)-ONp racemizes mainly through an enolization mechanism and that the cysteine side chain is responsible for this abnormal behavior. It is expected in practical peptide synthesis that coupling cysteine dipeptide active esters will yield an optically purer product than the coupling of cysteine active ester derivatives.

It was observed in our studies of racemization and coupling rates of amino acid and peptide active esters that Z-Gly-Cys(Bzl)-ONp (I) and the corresponding pentachlorophenyl ester racemize in the presence of NEt_3 much more slowly than predicted, by using the additivity principle, but couple at the expected rates.[2] The fast racemization of *S*-benzylcysteine active ester derivatives in the presence of a tertiary base had been reported earlier,[3] and it was shown that Z-Cys(Bzl)-OPcp and the corresponding ONp ester racemize through α-hydrogen abstraction and, furthermore, via the isoracemization mechanism.[4,5] Glycyl dipeptide active ester derivatives racemize about 50–100 times faster than the corresponding amino acid active ester derivatives;[2,6] therefore, it was expected that glycylcysteine active ester derivatives would also racemize about 2 orders of magnitude faster than the corresponding monomers. It was found that both the monomer and dipeptide active esters of cysteine racemize practically at the same rate. The theory which is used to calculate racemization rate constants is based on the additivity principle.[2,7] The calculated racemization rate constant, k_r, of Z-Gly-Cys(Bzl)-ONp in THF with NEt_3 using the predictive factors,[2] is $38\,300 \times 10^{-6}$ M^{-1} s^{-1}, while the observed k_r is 704×10^{-6} M^{-1} s^{-1}, which is only 1.8 times faster than the k_r for the monomer Z-Cys(Bzl)-ONp. A similar discrepancy was observed for Z-Gly-Cys(Bzl)-OPcp, for which the calculated k_r value is 19.2 times larger than the experimental value. Since the theory behind the rate constant calculations is a form of linear free energy relationships,[2,7,8] a large deviation from the calculated rate constants indicates deviation from the constant reaction mechanism.

It was also noted that IR spectroscopy did not detect the 5(4*H*)-oxazolone formation during the courses of racemization of *N*-(carbobenzoxyglycyl)-*S*-benzyl-L-cysteine active esters. These facts led us to conclude that the racemization of *S*-benzylcysteine dipeptide active esters must proceed through the enolization mechanism and not by 5(4*H*)-oxazolone formation. Detailed investigation of the mechanism of racemization of Z-Gly-Cys(Bzl)-ONp will be reported here.

Discussion

A distinction between the 5(4*H*)-oxazolone and the enolization mechanism of racemization of C-activated peptide derivatives is the following: in the 5(4*H*)-oxazolone formation the rate-determining step does not involve the breaking of an α-carbon–hydrogen bond, while in the enolization mechanism the slow step is the removal of α-hydrogen.[9-11] An earlier report[12] demonstrated that the deuterium isotope effect can be used to distinguish between the two mechanisms on α-deuterated compounds: the 5(4*H*)-oxazolone mechanism should not show an isotope effect, while the enolization path is expected to give an isotope effect provided that the 5(4*H*)-oxazolone formation is the rate-determining step and that the rate of 5(4*H*)-oxazolone racemization is much faster than the rate of its ring opening. If these conditions can be justified,

(1) Part of the paper was presented at the 5th American Peptide Symposium, San Diego, CA, 1977, and the 15th European Peptide Symposium, Gdansk, Poland, 1978.

(2) Kovacs, J.; Cover, R. E.; Jham, G.; Hsieh, Y.; Kalas, T. "Proceedings of the Fourth American Peptide Symposium"; Walter, R., Meienhofer, J., Eds.; Ann Arbor Science: Ann Arbor, MI, 1975; p 317.

(3) (a) Kovacs, J.; Mayers, G. L.; Johnson, R. H.; Cover, R. E.; Ghatak, U. R. *J. Org. Chem.* **1970**, *35*, 1810. (b) Kovacs, J.; Mayers, G. L.; Johnson, R. H.; Ghatak, U. R. *J. Chem. Soc., Chem. Commun.* **1968**, 1066. (c) Kovacs, J.; Mayers, G. L.; Johnson, R. H.; Cover, R. E.; Ghatak, U. R. *Ibid.* **1970**, 53. (d) Liberek, B. *Tetrahedron Lett.* **1963**, 925.

(4) (a) Mayers, G. L.; Kovacs, J. *J. Chem. Soc. D* **1970**, 1145. (b) Kovacs, J.; Cortegiano, H.; Cover, R. E.; Mayers, G. L. *J. Am. Chem. Soc.* **1971**, *93*, 1541.

(5) Kovacs, J.; Davis, E. J.; Johnson, R. H.; Cortegiano, H.; Roberts, J. E. "Proceedings of the Third American Peptide Symposium"; Meienhofer, J., Ed.; Ann Arbor Science: Ann Arbor, MI, 1972; p 359.

(6) Kovacs, J.; Holleran, E. M.; Hui, K. Y. *J. Org. Chem.* **1980**, *45*, 1060.

(7) Rouvray, D. H. *Chem. Technol.* **1973**, *3*, 379. The additivity principle was used for prediction of properties of organic compounds as early as 1820 by Liebig, who recognized the homologous series; later van't Hoff applied it for the prediction of optical rotation.

(8) Chapman, B., Shorter, J., Eds. "Advances in Linear Free Energy Relationship"; Plenum Press: New York, 1972; p 11.

(9) Goodman, M.; McGahren, W. J. *Tetrahedron* **1967**, *23*, 2301.

(10) Williams, M. W.; Young, G. T. "Proceedings of the Fifth European Peptide Symposium"; Young, G. Y.; Ed.; Pergamon Press: London, 1963; p 119.

(11) Williams, M. W.; Young, G. T. *J. Chem. Soc.* **1964**, 3701. Antonovics, I.; Young, G. T. *Ibid.* **1967**, 595.

(12) Kemp, D. S.; Rebek, J. *J. Am. Chem. Soc.* **1970**, *92*, 5792.

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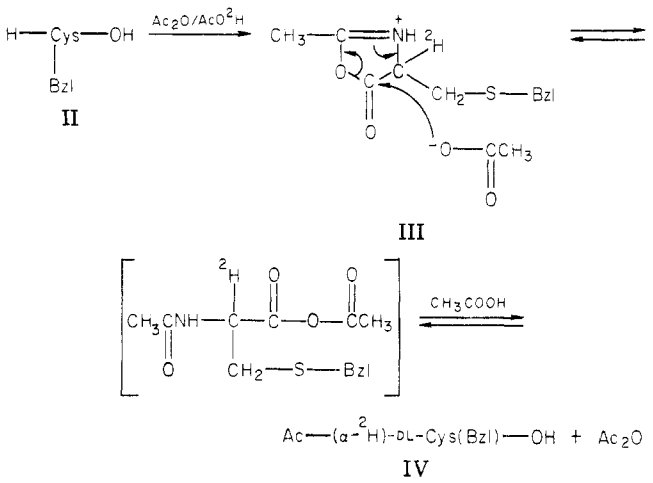
Scheme I



then by measuring $k_r^H/k_r^{^2H}$ we can distinguish the 5-(4*H*)-oxazolone mechanism from the enolization mechanism. In the present study *N*-(carbobenzoxyglycyl)-*S*-benzyl-L-cysteine *p*-nitrophenyl ester was used as a model compound.

Preparation of *S*-Benzyl-L-(α-^{2}H)cysteine (II).[13] It was expected that α-deuteration of *S*-benzyl-L-cysteine (II) can be achieved by treating it with an Ac_2O/AcOD mixture at elevated temperature.[14] The *N*-acetyl derivative formed first would give the 5(4*H*)-oxazolone III, which in the presence of AcOD would undergo deuterium exchange on the α-carbon atom by a racemization mechanism. Hydrolysis of III with D_2O would yield *N*-acetyl-*S*-benzyl-DL-cysteine (IV). However, when II was heated at reflux with acetic anhydride in acetic acid followed by evaporation of the solvent, crystalline *N*-acetyl-*S*-benzyl-DL-cysteine (IV) was obtained. In order to exclude the possibility that this intriguing result was caused by contamination by water, the experiment was repeated with 15 g of II under strictly anhydrous conditions, and again the racemic acid IV was isolated as the major product.

To study the effect of acetic acid on the 5(4*H*)-oxazolone of *N*-acetyl-*S*-benzylcysteine, we prepared III from Ac-Cys(Bzl)-OH with DCC. When III was dissolved in freshly prepared HOAc and the solution was left in a desiccator at room temperature, IV crystallized out of the reaction mixture several hours later. The precipitate IV was filtered, and the filtrate was distilled; one fraction was mainly acetic anhydride (IR, gas chromatography).

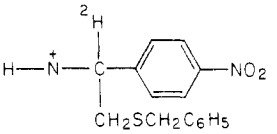
In view of this observation, it was concluded that the azlactone ring was opened by HOAc with the concomitant formation of acetic anhydride as shown by Scheme I.

The 5(4*H*)-oxazolone III, which was prepared by dehydration of Ac-DL-Cys(Bzl)-OH with DCC, upon cooling in a dry ice bath crystallized but melted at room temperature. For the deuteration process, the oily III was dissolved in 20 equiv of freshly prepared ^{2}HOAc and treated with 2 equiv of Et_3N, which was added to facilitate the enolization and therefore the deuterium exchange of the 5(4*H*)-oxazolone. At the end of the deuteration, an equivalent amount of CF_3COO^2H was added to the solution to neutralize the Et_3N, when *N*-acetyl-*S*-benzyl-DL-(α-^{2}H)cysteine crystallized from the solution in good yield. It was subsequently resolved in the presence of cobalt divalent ions with acylase I[14-16] to give *S*-benzyl-L-(α-^{2}H)cysteine.

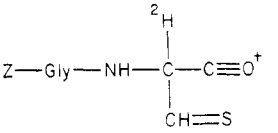
The above observations point to the following mechanisms: the 5(4*H*)-oxazolone III, upon treatment with ^{2}HOAc and Et_3N, was partially converted to its enol form by the base and partially into free acid and acetic anhydride. In this equilibrium system the deuteration can be completed. As the deuterium-exchange reaction was quenched by the addition of CF_3COO^2H, the enolate was converted into its keto form, which was then opened by ^{2}HOAc, and the deuterated free acid IV crystallized from the solution as shown in Scheme II.

Syntheses and Racemization Studies of *N*-Carbobenzoxy-*S*-benzyl-L-(α-^{2}H)cysteine *p*-Nitrophenyl Ester (VI) and *N*-(Carbobenzoxyglycyl)-*S*-benzyl-L-(α-^{2}H)cysteine *p*-Nitrophenyl Ester (VIII). The synthesis of the Z-Gly-(α-^{2}H)-L-Cys(Bzl)-ONp is outlined in Scheme III. *N*-Carbobenzoxy-*S*-benzyl-L-(α-^{2}H)cysteine (V) was prepared from α-deuterated cysteine II and then esterified with *p*-nitrophenol by using the DCC method to give *N*-carbobenzoxy-*S*-benzyl-L-(α-^{2}H)cysteine *p*-nitrophenyl ester VI.

The deuterium content of Z-(α-^{2}H)-L-Cys(Bzl)-ONp was calculated from the mass spectrum by using the *m/e* 332/331 peak abundance ratio after correcting for the ^{13}C contributions,[17] and the compound was found to be nearly 100% deuterated. The deuterium-containing *m/e* 332 fragment is believed to have the following structure:



The dipeptide active ester Z-Gly-(α-^{2}H)-L-Cys(Bzl)-ONp (VIII) was prepared from Z-Gly-OH and HBr·H-(α-^{2}H)-L-Cys(Bzl)-ONp (VII) by using the mixed anhydride coupling procedure. The deuterium content of VIII was 77% as determined by mass spectrometry using the *m/e* 294 fragment[17] which is believed to have the following structure:



It is noteworthy that the 60-MHz NMR spectra of the active ester salt VII and the dipeptide active ester VIII did not show the presence of α-protons; both VII and VIII gave the same melting points as the corresponding undeuterated samples.[18,22,29] The possibility of some racemization and therefore the loss of deuterium during the preparation of VII and VIII cannot be ruled out.

Racemization of optically pure active ester VI in THF with 7 equiv of Et_3N gave a $k_r^{^2H} = 52 \times 10^{-6}$ M^{-1} s^{-1} while the racemization rate constant of undeuterated VI[3b] was $k_r^H = 394 \times 10^{-6}$ M^{-1} s^{-1}. The ratio of $k_r^H/k_r^{^2H}$ gave an isotope effect of 7.5, as expected for the enolization mechanism. The racemate Z-(α-^{2}H)-DL-Cys(Bzl)-ONp which was isolated after the racemization was 94% completed, and the deuterium content was 64% as determined by mass spectrometry using the *m/e* 332 ion. The sole

(13) Recently, *S*-benzyl-L-(α-^{2}H)-cysteine was prepared by total synthesis followed by enzymatic resolution: Upson, D. A.; Hruby, V. J. *J. Org. Chem.* **1976**, *41*, 1353.

(14) Greenstein, J. P.; Winitz, M. "Chemistry of the Amino Acids"; Wiley: New York, 1961; p 1921.

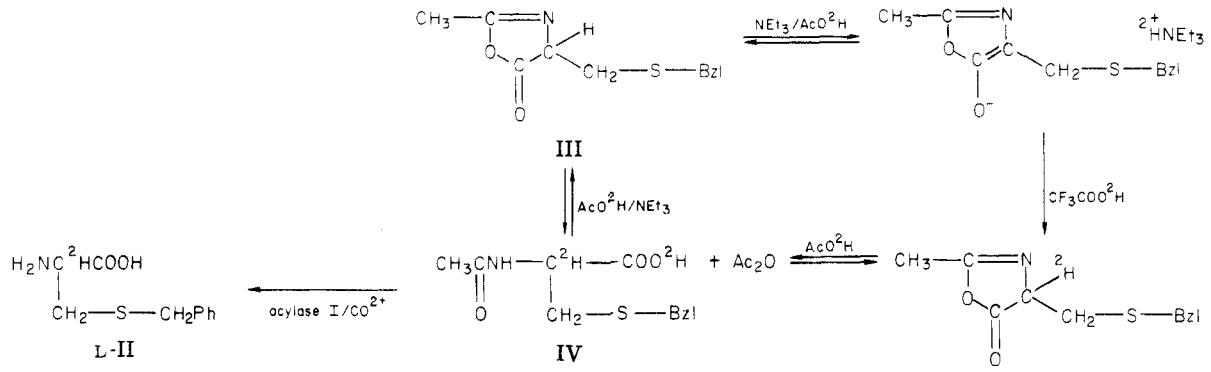
(15) Leukart, O.; Caviezel, M.; Eberle, A.; Escher, E.; Tun-Kyi, A.; Schwyzer, R. *Helv. Chim. Acta* **1976**, *59*, 2181.

(16) Marshall, R.; Birnbaum, S. M.; Greenstein, J. P. *J. Am. Chem. Soc.* **1956**, *78*, 4636.

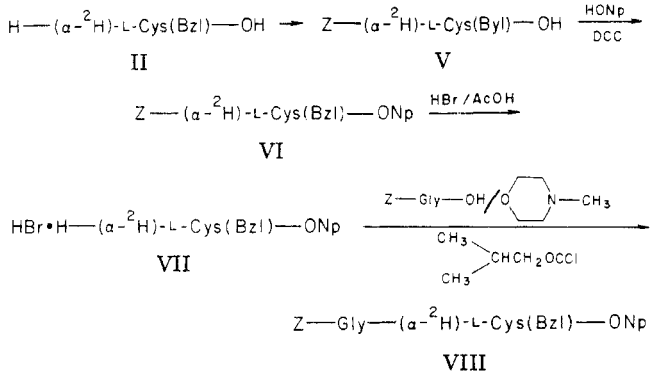
(17) Bieman, K. "Mass Spectrometry: Organic Chemical Applications"; McGraw-Hill: New York, 1962; Chapter 5.

(18) Macleren, J.; Savige, W. E.; Swan, J. M. *Aust. J. Chem.* **1958**, *11*, 360.

Scheme II



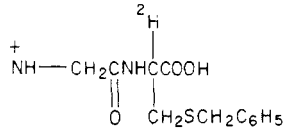
Scheme III



proton source in the medium was amide hydrogen, and therefore this value indicates α-deuterium and amide proton exchange during the course of racemization. The fact that more than 50% of deuterium was retained gives further support of isoracemization of *S*-benzylcysteine active ester derivatives.[4,5]

When dipeptide active ester VIII was racemized in the presence of 7 evuiv of Et_3N in THF, $k_r^{2H} = 335 \times 10^{-6}$ M^{-1} s^{-1} was obtained. The k_r^H of the undeuterated dipeptide active ester determined under the same conditions was 704×10^{-6} M^{-1} s^{-1}. The ratio of k_r^H/k_r^{2H} indicated an isotope effect of 2.1. This isotope effect of 2.1 is much smaller than that for Z-(α-^{2}H)-L-Cys(Bzl)-ONp, which is 7.5. However, the deuterium content of dipeptide VIII was only 77%, and therefore a lower value was necessarily obtained: no corrections were made throughout this paper for less than 100% deuterium conetne in calculating the isotope effect. In addition, the α-deuterium content changes during racemization due to the N–H proton source which makes the calculations more difficult. The isotope effect of 2.1 demonstrates that the removal of α-hydrogen in the racemization of the dipeptide active ester derivative VIII is the rate-determining step under these conditioins and that the racemization proceeds by enolization, or the enolization is parallel with the 5(4*H*)-oxazolone formation.

Studies on the Synthesis, Racemization, and Coupling Rates of the 5(4*H*)-Oxazolones of *N*-(Carbobenzoxyglycyl)-*S*-benzyl-L-cysteine and of *N*-(Carbobenzoxyglycyl)-*S*-benzyl-L-(α-^{2}H)cysteine. *N*-(Carbobenzoxyglycyl)-*S*-benzyl-L-cysteine (IX) was prepared by coupling of *N*-carbobenzoxyglycine pentachlorophenyl ester with *S*-benzyl-L-cysteine. Alternately, the dipeptide-free acid IX was prepared by saponification[18] of *N*-(carbobenzoxyglycy)-*S*-benzyl-L-cysteine methyl ester (X), which in turn was obtained by coupling of *N*-carbobenzoxyglycine and *S*-benzyl-L-cysteine methyl ester hydrochloride by using the mixed anhydride method. Similarly, *N*-(carbobenzoxyglycyl)-*S*-benzyl-L-(α-^{2}H)cysteine (XI) was synthesized by reaction of Z-Gly-OPcp and α-deuterated *S*-benzyl-L-cysteine (II). The deuterium content of XI was 71% as determined by mass spectrometry using the *m/e* 268 fragment, and it had the same melting point as the undeuterated sample.



Attempts to prepare 5(4*H*)-oxazolone of *N*-(carbobenzoxyglycyl)-*S*-benzyl-L-cysteine (XII) hydrochloride from the dipeptide-free acid IX and PCl_5 was unsuccessful; this procedure was used for the preparation of 5(4*H*)-oxazolone from Z-Phe-OH.[19]

Oxazolone XII was prepared from IX and 25% excess of DCC in 2 min at 23 °C. The reaction was quenched by addition of anhydrous ether and filtered into a large excess of petroleum ether precooled to −70 °C under a dry nitrogen atmosphere, and crystalline 5(4*H*)-oxazolone XII precipitated out of the solution. The 5(4*H*)-oxazolone racemizes rapidly at room temperature, and therefore it was used immediately after preparation for kinetic studies. The highest optical purity of XII prepared this way was 73%. The deuterated 5(4^2*H*)-oxazolone of *N*-(carbobenzoxyglycyl)-*S*-benzyl-L-(α-^{2}H)cysteine (XIII) was similarly prepared as indicated in Scheme IV.

The minimal optical purity of the 5(4*H*)-oxazolone was assessed by converting it into the corresponding hydrazide, Z-Gly-L-Cys(Bzl)-$NHNH_2$ (XIV), with anhydrous hydrazine. Hydrazine, an α-nucleophile, can open the 5(4*H*)-oxazolone ring without causing significant amount of racemization.[20] Optically pure hydrazide XIV was prepared from the dipeptide methyl ester X and hydrazine hydrate.

Both 5(4*H*)-oxazolone XII and 5(4^2*H*)-oxazolone XIII were found to lose their optical activity rapidly in tetrahydrofuran at room temperature. The autoracemization rate constant of XII in THF was 1015×10^{-6} s^{-1} ($c = 0.125$ M). If the 5(4*H*)-oxazolone itself is considered as the base, then the first-order rate constant divided by the base concentration gives the second-order racemization rate constant, $k_r = 8147 \times 10^{-6}$ M^{-1} s^{-1}. For the deuterated 5(4^2*H*)-oxazolone XIII the autoracemization rate constant was 365×10^{-6} s^{-1} ($c = 0.129$ M) and was 2832×10^{-6} M^{-1} s^{-1} for second-order reaction. These values indicate an isotope effect of $k_r^H/k_r^{2H} = 2.8$–2.9. The rate of autoracemization of XII in THF was greatly reduced at −70 °C: a 20% loss of optical purity of a THF solution of XII

(19) Jones, J. H.; Witty, M. J. *J. Chem. Soc., Chem. Commun.* **1977**, 281.
(20) Goodman, M.; Glaser, C. B. *J. Org. Chem.* **1970**, *35*, 1954.

stored at −70 °C was observed over a period of 3 days. The very large temperature coefficient for the 5(4*H*)-oxazolone racemization also supports previous observations of Anderson et al.[21] that the extent of racemization of C-activated peptides such as mixed anhydrides, where racemization is 5(4*H*)-oxazolone mediated, can be minimized at low temperatures.

The racemization of oxazolone XII and XIII with Et_3N in THF was instantaneous. However, the racemization rates of the two oxazolones with *N*-methyl morpholine were measurable in THF solution: the second-order rate constant was 1.11 M^{-1} s^{-1} for XII and 0.289 M^{-1} s^{-1} for XIII, which gave an isotope effect of 3.83.

The rate of coupling of XII with valine methyl ester in THF was measured by monitoring the rate of disappearance of the 5(4*H*)-oxazolone peak, which absorbs at 1837.7 cm^{-1}, using the IR technique. The second-order coupling rate constant k_c was found to be 8.5×10^{-2} M^{-1} s^{-1}.

From the above observations it was concluded that the coupling rate of XII is slow relative to the rate of racemization by Et_3N: that is, $k_r \gg k_c$.

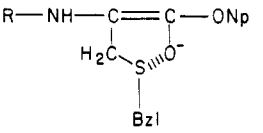
It was of great interest to investigate the rate of racemization of 5(4*H*)-oxazolone XII in the presence of valine methyl ester. The racemization rate during coupling was estimated from the data obtained on a Cary 60 spectropolarimeter. It was observed that an equimolar mixture of the diastereomeric tripeptides, Z-Gly-L-Cys(Bzl)-L-Val-OMe and Z-Gly-D-Cys(Bzl)-L-Val-OMe (XVI), gave practically zero rotation in THF.[22] The α values of the 5(4*H*)-oxazolone, H-Val-OMe and the LL and DL tripeptides were used for the calculations. The epimeric tripeptides LL and DL are formed in a ratio of 48% and 52%, respectively, as determined by HPLC. The calculated second-order racemization rate constant was 0.34 M^{-1} s^{-1}, and this value is substantially greater than k_c: that is, $k_r = 4k_c$. The racemization rate constant for the 5(4^2H)-oxazolone XIII was 0.14 M^{-1} s^{-1}, and an isotope effect of 2.6 was obtained.

Conclusion

The experimental results indicate that α-hydrogen abstraction is the rate-determining step in the racemization of Z-Gly-L-Cys(Bzl)-ONp, and the rate of racemization of the 5(4*H*)-oxazolone of *N*-(carbobenzoxyglycyl)-*S*-benzyl-L-cysteine greatly exceeds its rate of ring opening.

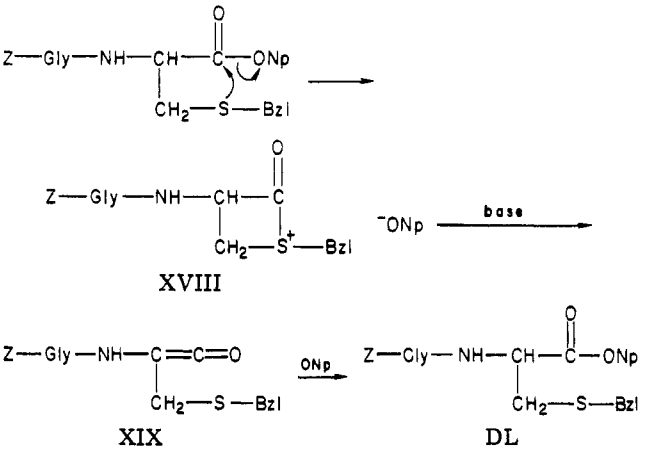
It was concluded that *N*-(carbobenzoxyglycyl)-*S*-benzylcysteine active esters racemize mainly through the enolization mechanism and that the *S*-benzyl cysteine side chain is responsible for this abnormal behavior. The only known dipeptide derivative which racemizes by enolization in the presence of Et_3N is *N*-(carbobenzoxyglycyl)-phenylalanine azide.[23] However, this racemization mechanism is carboxyl activation dependent since the racemization during the coupling of the Anderson dipeptide by other C-activated derivatives proceeds through the 5(4*H*)-oxazolone mechanism.[23]

A reasonable explanation for the abnormal behavior of Z-Gly-Cys(Bzl)-ONp in racemization is the following: the *S*-Bzl side chain makes the α-hydrogen so acidic that it can be removed directly without going through the 5-(4*H*)-oxazolone intermediate; that is, the energy of activation is lower for the enolization mechanism. Model studies show that in the enolate XVII, the sulfur is close



XVII

to the negatively charged oxygen. In this conformation, the d orbitals of sulfur likely will overlap with the p orbital of oxygen and by doing this will substantially increase the stability of the enolate which will be favored over the 5(4*H*)-oxazolone. The fast racemization of Z-Cys(Bzl)-ONp can also be more rationally explained by this quasi-five-membered ring, XVII, than by overlapping the p orbital of the α-carbon and the d orbitals of sulfur, as proposed by Jones,[24] which would form a quasi-three-membered ring. An alternate interpretation of the above results could be the following: the sulfur is participating as a nucleophile to form an *S*-benzyl β-thiolactone sulfonium ion XVIII.



XVIII XIX DL

This unstable intermediate would open to a ketene and thus racemize. The ketene would rapidly collapse back with the ONp group to regenerate the original compound. However, in this pathway the ketene intermediate rules out the isoracemization mechanism which was proved to operate in the racemization of the monomer.

The above data are useful in practical peptide synthesis. For example, it is expected that coupling of cysteine dipeptide active esters will yield optically purer products than coupling of cysteine active ester derivatives. Furthermore, the penultimate amino acid residue, which is racemization prone when the 5(4*H*)-oxazolone mechanism is operative,[25] is not expected to racemize when peptide coupling is conducted at carboxyl-terminal cysteine residues by active ester activation.

Experimental Section

All N-protected amino acids were purchased from Beckman Instruments, Inc.; the purity was checked by TLC and melting point, and they were recrystallized if necessary. Solvents as well as other reagents used in kinetic studies were purified according to the procedures described in ref 26 and stored under anhyrous conditions. Freshly distilled valine methyl ester was used for couplings. Melting points were determined with Thomas-Hoover apparatus and are reported uncorrected. The kinetics of racemization were studied either with a Rudolph photoelectric polarimeter Model 200 S-340-8006 or a Cary 60 recording spectro-

(21) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. W. *J. Am. Chem. Soc.* **1967**, *89*, 5012.
(22) The undeuterated dipeptide VIII and the epimeric tripeptides were prepared by K. Y. Hui.
(23) Kemp, D. S.; Want, S. W.; Busby, G.; Hugel, G. *J. Am. Chem. Soc.* **1970**, *92*, 1043.
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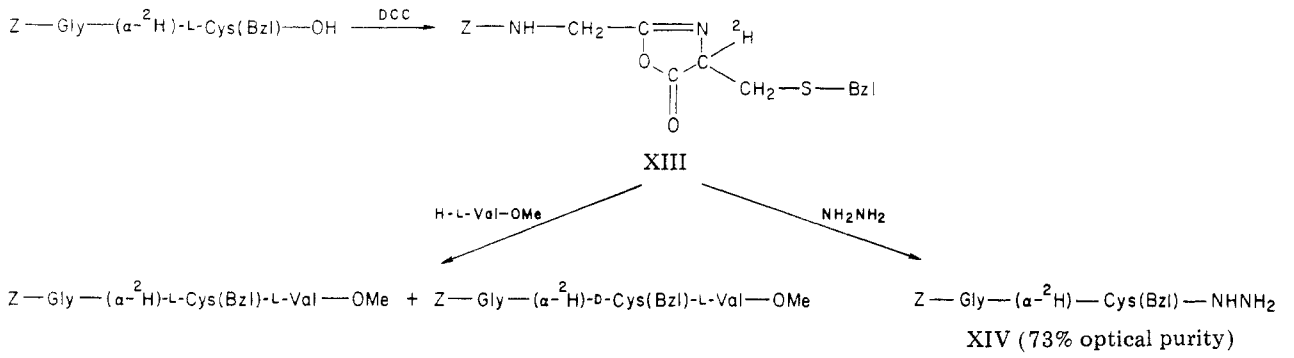
Scheme IV



polarimeter. Aminolysis rate studies were made on a Perkin-Elmer spectrophotometer (Model 283) with a time-drive setting for fast reactions or on a Beckman IR-8 spectrophotometer for slower couplings. All kinetic studies were carried out in a constant-temperatures room (23 ± 1 °C). NMR spectra were recorded with a Varian T-60 spectrometer.

***N*-Acetyl-*S*-benzyl-DL-(α-²H)cysteine (VI).** *N*-Acetyl-*S*-benzyl-DL-cysteine[14] (10.14 g, 40 mmol) was suspended in 50 mL of anhydrous THF and treated with DCC (8.24 g, 40 mmol). The reaction mixture was stirred at room temperature for 2 h. The precipitated DCU was removed by filtration; the total yield of DCU was 8.42 g (94%). The IR spectrum (THF) of the filtrate showed peaks at 5.5 and 5.9 μm. The solution was then evaporated to dryness under reduced pressure. The oily residue was dissolved in 20 equiv of freshly prepared ²HOAc (approximately 48 mL, obtained by refluxing 39 mL of Ac_2O and 7.3 mL of deuterium oxide) and treated with 11.2 mL of Et_3N (sodium dried, 80 mmol) under cooling. The solution was left in a desiccator for 2 h. The solvent was removed under reduced pressure, and the residue was dissolved in freshly prepared ²HOAc (20 equiv). The deuteration procedure was repeated two more times. After the third deuteration the residue was dissolved in 25 mL of freshly prepared ²HOAc, and 80 mmol of CF_3CO_2 ²H (approximately 7 mL, prepared by mixing 6 mL of trifluoroacetic anhydride and 0.73 mL of deuterium oxide in a cold bath under a dry nitrogen atmosphere and used immediately) was added. The deuterated acid crystallized from the solution upon standing in a desiccator overnight; yield 7.42 g. The filtrate was treated with 15 mL of deuterium oxide, and an additional 1.5 g of the deuterated acid was isolated; overall yield 84%. The crude product was recrystallized from warm acetone: mp 156–157 °C; NMR (TFA) 2.5 (s, 3 H), 3.4 (s, 2 H), 4.4 (s, 2 H), 8 (s, 5 H) ppm. For the undeuterated *N*-acetyl-*S*-benzyl-DL-cysteine: mp 157 °C;[14] NMR (TFA) 2.5 (s, 3 H), 3.4 (d, J = 6 Hz, 2 H), 4.4 (s, 2 H), 5.48 (m, 1 H), 8 (s, 5 H) ppm.

***S*-Benzyl-L-(α-²H)cysteine (II).** To a suspension of *N*-acetyl-*S*-benzyl-DL-(α-²H)cysteine (6 g, 23.5 mmol) in 400 mL of water was added concentrated NH_4OH dropwise until a clear solution was obtained (pH 9.2); the pH was then adjusted to 7.5 with 2 N HCl. The solution was kept at 37–37.2 °C for 20 min, and then 25 mg of $CoAc_2$ and 12 mg of acylase I (1000 U/mg)[15,16] were added. The solution was incubated at 37 °C for 20 h. The pH of the solution dropped to 5.5 and was adjusted to 7.5, and another 10 mg of acylase I was added; the solution was incubated for an additional 20 h. The solution was heated to 50 °C for 20 min, decolorized with charcoal, and filtered. The clear filtrate was adjusted to pH 5–5.5 and evaporated under reduced pressure to 75 mL when II started to crystallize out from the solution. After refrigeration overnight it was filtered and washed with cold water, acetone, and ether; yield 2 g (80%). The crude product was dissolved in 2 N HCl and precipitated by adjusting the pH to 5.5 with diluted NH_4OH: mp 213–214 °C; $[\alpha]^{23}_D$ +26° (*c* 1, 1 N NaOH); NMR (TFA) 3.5 (s, 2 H), 4.4 (s, 2 H), 8 (br s, 7 H) ppm. lit[14] mp 212–213 °C and $[\alpha]^{23}_D$ +25.5° (*c* 1, 1 N NaOH) for the undeuterated compound. NMR spectrum of the undeuterated II (TFA): 3.17 (d, 1 H, J = 4 Hz), 3.28 (s, 1 H), 3.85 (s, 2 H), 4.26 (br m, 1 H), 7.29 (br s, 2 H), 7.39 (s, 5 H) ppm.

***N*-Carbobenzoxy-*S*-benzyl-L-(α-²H)cysteine (V).** This compound was prepared according to the procedure reported for the undeuterated compound.[27] The crude product was recrystallized from $CHCl_3$–petroleum ether: yield 1.23 g (71%); mp 94–96 °C; $[\alpha]^{23}_D$ −49° (*c* 2, acetone); lit mp[27] (for the undeuterated compound) 93–95 °C.

***N*-Carbobenzoxy-*S*-benzyl-L-(α-²H)cysteine *p*-Nitrophenyl Ester (VI).** The preparation of the undeuterated compound[28] by using the DCC procedure was modified as follows: after DCU was removed from the reaction mixture by filtration, the filtrate was evaporated to dryness under reduced pressure. The oily residue was dissolved in ether, petroleum ether was added to the cloudy point, and the solution was warmed to give a clear solution. The product crystallized out when the solution was slowly cooled to room temperature. The crude product was recrystallized once from warm ether and then from ethanol: yield 1 g (71.4%); mp 94–95 °C; $[\alpha]^{23}_D$ −41.5° (*c* 1, DMF); $[\alpha]^{23}_D$ −30.0 (*c* 1.45, THF); lit[28] mp (for the undeuterated compound) 93–94 °C, $[\alpha]^{23}_D$ −42° (*c* 1, DMF). The mass spectrum showed peaks at m/e 376 (M^+ − 91) and 332 (M^+ − 135). The deuterium content of this compound determined by using the m/e 332 ion was nearly 100%.

***S*-Benzyl-L-(α-²H)cysteine *p*-Nitrophenyl Ester Hydrobromide (VII).** *N*-Carbobenzoxy-*S*-benzyl-L-(α-²H)cysteine *p*-nitrophenyl ester (VI; 1.17 g, 2.5 mmol) was treated with 5 mL of 45% HBr/HOAc in an ice bath with stirring. After CO_2 evolution ceased, a clear yellow solution was obtained. The salt was precipitated with 150 mL of anhydrous ether, filtered, washed thoroughly with ether, and recrystallized from methanol–ether: yield 0.83 g (80%); mp 155–155.5 °C; $[\alpha]^{23}_D$ +14.25° (*c* 2.04, EtOH); NMR (Me_2SO-d_6) 2.7 (d, 2 H, J = 2 Hz), 3.5 (s, 2 H), 3.65 (br s, 2 H), 4.23 (s, 2 H), 7.95 (s, 5 H), 8.2 (d, 2 H, J = 8 Hz), 9.1 (d, 2 H, J = 8 Hz) ppm; Literature[29] values for the undeuterated sample: mp 155–155.5 °C; $[\alpha]^{23}_D$ −14.6° (*c* 2.03, EtOH). NMR of *S*-benzyl-DL-cysteine *p*-nitrophenyl ester hydrobromide (Me_2SO-d_6) 2.7 (d, 2 H, J = 2 Hz), 3.5 (s, 2 H), 3.64 (br s, 2 H), 4.23 (s, 2 H), 7.95 (s, 5 H), 8.2 (d, 2 H, J = 8 Hz), 9.1 (d, 2 H, J = 8 Hz) ppm.

***N*-(Carbobenzoxyglycyl)-*S*-benzyl-L-(α-²H)cysteine *p*-Nitrophenyl Ester (VIII).** Z-Gly-OH (0.334 g, 1.6 mmol) was dissolved in 5 mL of THF and then cooled to −20 °C, and 0.17 mL of *N*-methylmorpholine and 0.22 mL of isobutyl chloroformate (1.6 mmol each) were added. The solution was stirred for 10 min, finely powdered active ester salt VII (0.663, 1.6 mmol) was suspended in the solution, and 0.17 mL of *N*-methylmorpholine (1.6 mmol) in 10 mL of THF was added dropwise over a period of 1 h with stirring at −15 to −20 °C. The reaction mixture was filtered and evaporated to dryness. The oily residue was dissolved in 25 mL of EtOAc, washed with water (3 × 10 mL), dried over Na_2SO_4, and concentrated under reduced pressure to about 3 mL. A small amount of white solid, formed after refrigeration, was removed by filtration, the filtrate was evaporated to near dryness, 5 mL of cold absolute ethanol was added, and the solution was refrigerated for several hours: yield 0.797 g (96%); mp 123–124 °C; $[\alpha]^{23}_D$ −35 (*c* 0.56, DMF); NMR ($CDCl_3$) 3.2 (s, 2 H), 4 (s, 2 H), 4.2 (d, 2 H, J = 6 Hz), 5.4 (s, 2 H), 7.7 (d, 2 H, J = 8 Hz), 7.9 (s, 10 H), 8.8 (d, 2 H, J = 8 Hz) ppm; mass spectrum, m/e 250 (M^+ − 274), 294 (M^+ − 230). The deuterium content calculated from

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(28) Bodanszky, M.; du Vigneaud, V. *J. Am. Chem. Soc.* **1959**, *81*, 2504.
(29) Goodman, M.; Steuben, K. C. *J. Am. Chem. Soc.* **1959**, *81*, 3980.

its mass spectrum by using m/e 294 ions was 77%.

N-Carbobenzoxy-S-benzyl-DL-(α-^{2}H)cysteine p-Nitrophenyl Ester. N-Carbobenzoxy-S-benzyl-L-(α-^{2}H)cysteine p-nitrophenyl ester (0.234 g, 0.25 mmol) was dissolved in 4 mL of anhydrous THF in a drybox under a dry nitrogen atmosphere, Et_3N (0.49 mL, 3.5 mmol) was added, and the solution was diluted to 5 mL with THF. The active ester was allowed to racemize in a desiccator at room temperature for 28 h. The solution was neutralized with 3.5 mmol of CF_3COO^2H (freshly prepared by mixing 0.04 mL of D_2O and 0.3 mL of trifluoroacetic anhydride) under a dry nitrogen atmosphere and evaporated to dryness under reduced pressure. The residue was dissolved in 15 mL of EtOAc, washed with water until the organic phase was neutral, dried over Na_2SO_4, and concentrated to about 3 mL under reduced pressure. The crystallization of the racemate started at room temperature, the solution was refrigerated overnight, and the solid mass was triturated with a small amount of ether and filtered: yield 0.169 g (72%); mp 110–111 °C; $[\alpha]^{23}_D$ −2.66° (c 1.015, DMF); 94% racemized; IR (KBr) 5.6 μm. The mass spectrum indicated 64% deuterium content using the m/e 332 ion.

N-Carbobenzoxy-S-benzyl-DL-cysteine p-Nitrophenyl Ester.[22] The undeuterated compound was prepared similarly in 70% yield: mp 109–110 °C; $[\alpha]^{18}_D$ 0.02 (c 0.5, DMF).

N-(Carbobenzoxyglycyl)-S-benzyl-L-cysteine p-Nitrophenyl Ester.[22] The undeuterated Z-Gly-L-Cys(Bzl)-ONp was prepared from HBr·H-L-Cys(Bzl)-ONp and Z-Gly-OH according to the procedure described for the deuterated compound VIII. **HBr·H-L-Cys(Bzl)-ONp**[22] [mp 153–154 °C; $[\alpha]^{22}_D$ −8.6° (c 0.5, DMF)] was obtained from Z-L-Cys(Bzl)-ONp [mp 90–91 °C; $[\alpha]^{22}_D$ −44 (c 1, DMF)] by the same procedure as VII was prepared. Similarly, **TFA·H-L-Cys(Bzl)-ONp**[22] [mp 109–110 °C; $[\alpha]^{22}_D$ +1.4° (c 0.5, DMF)] was coupled with Z-Gly-OH in 92% yield. The trifluoroacetate was prepared the usual way from **Boc-L-Cys(Bzl)-ONp**:[30] mp 94–95 °C; $[\alpha]^{22}_D$ −39.3° (c 1, DMF); lit.[30] mp 95–96 °C; $[\alpha]_D$ −37.5° (c 1.2, DMF). The crude dipeptide derivative was precipitated from ether–petroleum ether (1:1); mp 89–90 °C. This crude pruduct was dissolved in EtOAc and filtered, and filtrate was concentrated again. The product was crystallized from ether–petroleum ether (1:1): mp 123.5–124.5 °C; $[\alpha]^{22}_D$ −38 (c 0.5, DMF). This compound was prepared previously by using the mixed anhdydride procedure in 8% yield; mp 165–166.5 °C and $[\alpha]^{23}_D$ −29° (c 0.5, DMF) were reported.[31]

The racemic undeuterated dipeptide derivative N-(carbobenzoxyglycyl)-S-benzyl-DL-cysteine p-nitrophenyl ester was prepared from HBr·H-DL-Cys(Bzl)-ONp[22] [mp 174.5–175.5 °C; $[\alpha]^{23}_D$ +0.01° (c 0.5, DMF); obtained from Z-DL-Cys(Bzl)-ONp with HBr/AcOH] and Z-Gly-OH by using the mixed anhydride procedure described for the L isomer. The reaction mixture was concentrated under reduced pressure, ether–petroleum ether was added to the residue, and crystalline material was obtained; mp 119–120 °C. This compound was converted to the tripeptide with H-L-Val-OMe as described below.

N-(Carbobenzoxyglycyl)-S-benzyl-DL-cysteinyl-L-valine Methyl Ester.[22] Z-Gly-DL-Cys(Bzl)-ONp (0.5 mmol, 262 mg) was dissolved in absolute THF in a 5-mL volumetric flask. H-Val-OMe (0.5 mmol, 0.0656 mL) was added, and the flask was filled with THF to 5 mL. The mixture was allowed to stand at room temperature overnight. It was then evaporated to dryness, redissolved in 50 mL of EtOAc, washed five times with 0.1% sodium bicarbonate solution and then water until neutral, dried with anhydrous Na_2SO_4, filtered, and concentrated again. This concentrated crude product was diluted with a minimum amount of ether–petroleum ether (1:1) and then petroleum ether until the cloudy point and left at room temperature overnight. The crude crystal was washed with ether–petroleum ether (1:1): mp 110–112 °C; R_f 0.42 (benzene/EtOAc 1:1); yield 60%; $[\alpha]^{19}_D$ +30° (c 0.5, DMF), near zero in THF. Anal. Calcd for $C_{26}H_{33}O_6N_3S$: C, 60.56; H, 6.45. Found: C, 59.55; H, 6.21.

The HPLC chromatogram[32] of the epimeric tripeptide mixture was made on a Waters Associates Model ALC/GPC 244 liquid chromatograph with a C_{18} Bondpak/1 ft × 7 mm column and a linear gradient elution solvent system; acetonitrile/water containing 1% acetic acid was used, and the LL diastereomer gave a peak at 155 mm while the DL isomer appeared at 158.5 mm (15.5 and 15.85 min, respectively). As a control Z-Gly-L-Cys(Bzl)-L-Val-OMe was used, and it appeared at 154.5 mm. The peaks indicated the presence of 47.7% LL and 52.3% DL epimers for the crystallized material.

N-(Carbobenzoxyglycyl)-S-benzyl-L-cysteinyl-L-valine Methyl Ester.[22] The LL tripeptide was similarly prepared from Z-Gly-L-Cys(Bzl)-ONp and H-L-Val-OMe: 90% yield; mp 96–98 °C; $[\alpha]^{22}_D$ −30° (c 0.5, DMF). Anal. Calcd for $C_{26}H_{33}O_6N_3S$: C, 60.56; H, 6.45. Found: C, 59.97; H, 5.81.

N-(Carbobenzoxyglycyl)-S-benzyl-L-cysteine (IX). (a) S-Benzyl-L-cysteine (1.07 g, 5.06 mmol) was dissolved in a mixture of 5 mL of THF and 10 mL of water containing 0.71 mL of Et_3N (5.06 mmol). To this were added Z-Gly-OPcp (2.32 g, 5.06 mmol) and Et_3N (0.71 mL, 5.06 mmol) in 15 mL of THF. The clear solution was left at room temperature for 5 h. THF was removed under reduced pressure, the aqueous solution was diluted with more water, and the solid material was filtered and washed with EtOAc (2 × 15 mL). The solution was acidified with 2 N HCl, and the cloudy solution was extracted with EtOAc (2 × 20 mL). The combined EtOAc layers were washed with water, dried over Na_2SO_4, and evaporated to dryness under reduced pressure. The oily residue was crystallized from ether: mp 117–119 °C; yield 1.4 g (70%). The crude product was recrystallized from EtOAc: mp 119–120 °C; $[\alpha]^{23}_D$ −18° (c 1.29, THF); $[\alpha]^{25}_D$ −22.2° (c 1.192, EtOH); NMR (acetone-d_6) 2.8 (d, 1 H, J = 3 Hz), 2.9 (s, 1 H), 3.7 (s, 2 H), 3.9 (d, 2 H, J = 7 Hz), 4.8 (br m, 1 H), 5.05 (s, 2 H), 6.7 (br s, 1 H), 7.4 (s, 10 H), 8.05 (br s, 1 H), ppm; mass spectrum, m/e 311 (M^+ − 91), 267 (M^+ − 135), 250 (M^+ − 152). The preparation of this compound was reported earlier[18] by saponification of Z-Gly-L-Cys(Bzl)-OEt: mp 120–121 °C; $[\alpha]^{18}_D$ −24° (c 1.2, EtOH). In addition to the optically active compound, and equal amount of the racemate was also obtained by using fractional crystallization.

(b) N-(Carbobenzoxyglycyl)-S-benzyl-L-cysteine methyl ester (X; 0.412 g, 1 mmol) was dissolved in 5 mL of THF, 1.1 mL of NaOH was added, and the solution was left at room temperature for 4.5 h. THF and NaOH solution are not completely miscible; therefore, 2 mL of EtOH was added to the solution. After the solution was stirred for another 2 h, THF and EtOH were removed under reduced pressure, and the aqueous phase was diluted with 4 mL of water, washed with 3 mL of EtOAc, and acidified to pH 2. The cloudy solution was extracted with EtOAc (3 × 5 mL), and the combined organic layer was washed with water, dried over Na_2SO_4, and concentrated to a small volume under reduced pressure. The product was precipitated with ether: yield 0.28 g (70%); mp 118–119 °C; $[\alpha]^{23}_D$ −16.4 (c 1.3, THF). This rotation indicated 84% optical purity, which was substantially less racemization than that which occurred during the saponification of the corresponding ethyl ester.[18] A mixture melting point with the product obtained according to procedure a gave no depression. IR spectrum was superimposable with that obtained unser procedure a.

N-(Carbobenzoxyglycyl)-S-benzyl-(α-^{2}H)cysteine (XI). This compound was prepared by the procedure described in a for the undeuterated dipeptide above. The crude product was crystallized from ether and recrystallized from EtOAc: yield 0.689 g (72%); mp 119–120 °C; $[\alpha]^{23}_D$ −17.2° (c 1.3, THF); NMR (acetone-d_6) 3.0 (d, 2 H, J = 2 Hz), 3.83 (s, 2 H), 4.0 (d, 2 H, J = 7 Hz), 5.2 (s, 2 H), 6.4 (br s, 1 H), 6.7 (br s, 1 H), 7.5 (s, 10 H), 8.05 (br s, 1 H); mass spectrum, m/e 312 (M^+ − 91), 267 (M^+ − 136), 250 (M^+ − 153). The deuterium content of this compound determined by its mass spectrum by using the m/e 312 ion was 71%.

N-(Carbobenzoxyglycyl)-S-benzyl-L-cysteine Methyl Ester (X). Z-Gly-OH (3.14 g, 15 mmol) was dissolved in 70 mL of THF with stirring at room temperature, the solution was cooled to 0 °C, and 2.1 mL of Et_3N and 2.1 mL of isobutyl chloroformate (15 mmol each) were added. The solution was stirred in the cold for 10 min, and then S-benzyl-L-cysteine methyl ester hydrochloride (3.93 g, 15 mmol) and Et_3N (2.1 mL, 15 mmol) were added to it. The suspension was stirred at −10 to −5 °C for 2.5 h. The Et_3N hydrochloride was removed by filtration (4.1 g, 93%), and

(30) Beyerman, H. C.; Boers-Boonekamp, C. A. M.; van den Brink-Zimmermannova, H. Maasen *Recl. Trav. Chim. Pays-Bas* **1968**, *87*, 257.
(31) Stewart, F. H. C. *Aust. J. Chem.* **1966**, *19* (8), 1503.
(32) The HPLC chromatogram was made by Dr. S. I. Sallay at Purdue University, Fort Wayne, IN, and we wish to thank him for it.

the clear filtrate was evaporated to dryness under reduced pressure. The resulting oil was dissolved in 70 mL of EtOAc, and the solution was washed with 20 mL of water, 15 mL of saturated sodium bicarbonate solution, and portions of water (2 × 20 mL). The organic phase was dried over Na_2SO_4, and the product crystallized upon evaporation of the solution to dryness under reduced pressure. The dipeptide methyl ester was recrystallized from EtOAc: yield 4.79 g (78%); mp 98–101 °C; $[\alpha]^{23}_D$ −24.5° (*c* 1.6, THF). Further recrystallization from methanol and then from THF raised the melting point to 103–104 °C ($[\alpha]^{23}_D$ −28.3° (*c* 1.63, THF)). Anal. Calcd for $C_{21}H_{24}N_2O_5S$: C, 60.56; H, 5.81; N, 6.73. Found: C, 60.73; H, 5.81; N, 6.44.

***N*-(Carbobenzoxyglycyl)-*S*-benzyl-L-cysteine Hydrazide (XIV).** To a solution of 0.833 g of Z-Gly-L-Cys(Bzl)-OMe (X, 2 mmol) in 9 mL of methanol was added 0.16 mL of freshly distilled anhydrous hydrazine. The clear colorless solution was left in the dark at room temperature, and the hydrazide crystallized out. It was triturated with 1 mL of methanol and filtered: yield 0.69 g (83%); mp 149–150 °C; $[\alpha]^{23}_D$ −13.5° (*c* 2.04, DMF). Anal. Calcd for $C_{20}H_{24}N_4O_4S$: C, 57.68; H, 5.81; N, 13.45. Found: C, 57.41; H, 5.47; N, 13.25.

Another experiment was carried out with 1 mmol of X and 0.34 mL of hydrazine in 2 mL of DMF at room temperature for 24 h and gave similar results. At the end of the reaction the hydrazide was precipitated with water and recrystallized from THF and methanol: yield 36 mg (87%); mp 149–150.5 °C.

5(4*H*)-Oxazolone of *N*-(Carbobenzoxyglycyl)-*S*-benzyl-L-cysteine (XII). A stock solution of Z-Gly-L-Cys(Bzl)-OH (IX, 3 mmol) in 3 mL of THF was prepared, and an aliquot of 1 mL (0.302 g, 0.75 mmol) was added to 0.206 g of DCC (1 mmol) in 0.1 mL of THF with stirring at room temperature. The reaction was quenched after 2 min by dilution with 2 mL of anhydrous ether and subsequent cooling to −70 °C in dry ice. The reaction mixture was filtered into 30 mL of dry petroleum ether which had been precooled to −70 °C. All manipulations were carried out in a drybox under a dry nitrogen atmosphere. Crystalline XII precipitated out from the solution immediately. The supernatant was decanted, and the product was triturated with precooled petroleum ether three times and dried under high vacuum; yield 0.136 g (36%). The IR showed the characteristic peaks for the 5(4*H*)-oxazolone at (THF) 5.4, and 5.9 μm. Compound XII was crystalline at −70 °C but melted at room temperature. The isolated DCU weighed 0.162 g (72.3%). Due to its fast racemization tendency, the 5(4*H*)-oxazolone was used for kinetic studies immediately after preparation. The yield of XII prepared as described above ranged from 36% to 68%, and in each reaction 70–83% of DCU was collected.

5(4^2*H*)-Oxazolone of *N*-(carbobenzoxyglycyl)-*S*-benzyl-L-(α-^{2}*H*)cysteine (XIII) was prepared the same way as described above for the undeuterated compound.

The optical purity of XIII was determined by treating it with 1 equiv of anhydrous hydrazine at 0 °C in THF, and the resulting hydrazide was isolated by evaporation of the reaction mixture to dryness followed by trituration of the residue with warm ether. The crude product was recrystallized from THF–ether: yield 97%; $[\alpha]^{23}_D$ −9.85° (*c* 2.03, DMF). The minimal optical purity of 5-(4^3*H*)-oxazolone XIII was 73% as shown by the rotation of the hydrazide. Compounds XII and XIII prepared by the DCC method generally gave optical purity ranging from 50% to 73%.

Racemization Rate Studies of the 5(4*H*)-Oxazolone XII and 5(4^2*H*)-Oxazolone XIII. (a) With *N*-Methylmorpholine. Autoracemization of XII (0.0683 M in THF) was followed on a Cary 60 spectropolarimeter. When the solution showed a specific rotation of $[\alpha]^{23}_D$ −31.8° (*c* 2.33, THF), this indicated an optical purity of 23.4%. At this point 0.016 M optically pure XII and 0.0523 M racemic 5(4*H*)-oxazolone was present in the solution. *N*-Methylmorpholine (0.3 mL of a 0.5 M solution in THF) was added to the solution which actually contained 0.0536 M *N*-methylmorpholine and 0.016 M L-5(4*H*)-oxazolone, and the racemization was followed in the same cell. The plot of the logarithm of rotation vs. time gave a straight line, and the slope gave a pseudo-first-order rate constant of 0.0594 s^{-1} and a second-order rate constant of 1.11 M^{-1} s^{-1}. The racemization rate constant of XIII was similarly determined: the second-order rate constant with *N*-methylmorpholine (concentrations were 0.0183 M XIII and 0.0536 M *N*-methylmorpholine) was 0.289 M^{-1} s^{-1}, which gave an isotope effect of 3.83.

(b) With L-Valine Methyl Ester. A THF solution of XII (2.5 mL of a 0.1246 M solution, optical purity 49.8%) was allowed to racemize at room temperature until the observed rotation indicated an optical purity of approximately 16.6%. An equivalent amount of L-valine methyl ester (0.312 mmol, 0.125 M) was then added. The reaction mixture gave a specific rotation of −10.95° 11 s after the addition of H-Val-OMe, indicating a composition of 0.0256 M optically pure XII and 0.112 M valine methyl ester [calculated from the second-order coupling rate constant of the 5(4*H*)-oxazolone with H-Val-OMe which is $k_c = 8.5 \times 10^{-3}$ M^{-1} s^{-1}; 10.43% of the amine and of the 5(4*H*)-oxazolone should have been coverted into a pair of diastereomeric tripeptides, assuming that the L- and D-5(4*H*)-oxazolone couple equally fast]. Two more points were arbitrarily chosen at 16 and 21 s after the introduction of the nucleophile. These three points gave a straight line when the logarithm of rotation was plotted against time. At the end of the calculated coupling time the solution showed practically zero rotation, and approximately equal amounts of the epimeric tripeptides Z-Gly-L-Cys(Bzl)-L-Val-OMe and Z-Gly-D-Cys-(Bzl)-L-Val-OMe were present as determined by HPLC.[32] The second-order rate constant was calculated from the pseudo-first-order rate constant/H-Val-OMe concentration ratio and was 0.346 M^{-1} s^{-1}. The base concentration used for the calculation was 0.106 M, which is the average of the concentrations of H-Val-OMe at 11, 15, and 21 s. The extent of racemization of XII by valine methyl ester was approximately 64% during the first 11 s, as it was estimated from the rotation drops.

The deuterated 5(4^2*H*)-oxazolone XIII was subjected to the same experiment, and it was found that 12.5% of the initial amount of XIII was racemized by H-Val-OMe during the first 48 s. Since the deuterated species racemize considerably slower than the protio analogue, the treatment of the kinetic data was further complicated by the fact that an appreciable larger amount of XIII had reacted with valine methyl ester, and as a result an indistinguishable fraction of negative rotation of their coupling product contributed to the overall rotation of the reaction mixture. For this reason, two sets of data were chosen over a short span of time and used for the calculation of the racemization rate constant. The first set of three points at 107, 112, and 117 s were plotted, and the logarithms of rotations against time gave a straight line. The base concentration was the average of the concentrations of H-Val-OMe at the three ponts and was found to be 0.058 M which is equivalent to 3.02 times the concentration of XIII at 107 s. k_r^D is equal to 6942×10^{-6} s^{-1} for the first-order reaction and 0.12 M^{-1} s^{-1} for the second-order reaction. The second set of four points were selected from 147 to 162 s, and $k_r^D = 0.14$ M^{-1} s^{-1} was obtained. The base concentration was the average valine methyl ester concentration between 147 to 167 s and was found to be 3.42 times the concentration of XIII at 147 s. The average of the two rate constants, $k_r^D = 0.13$ M^{-1} s^{-1}, is reported here, and an isotope effect of 2.6 was obtained.

The racemized 5(4*H*)-oxazolone was analyzed and gave correct C, H, and N values. Anal. Calcd for $C_{20}H_{20}N_2O_4S$: C, 62.48; H, 5.24; N, 7.30. Found: C, 62.24; H, 5.52; N, 7.55. The tripeptide Z-Gly-(α-^{2}H)-Cys(Bzl)-L-Val-OMe, obtained by coupling of the 5(4^2*H*)-oxazolone XIII and H-Val-OMe, was a mixture of the LL and DL diastereomers. Anal. Calcd for $C_{26}H_{33}O_6N_3S$: C, 60.56; H, 6.45; N, 8.16. Found: C, 60.21; H, 6.87; N, 7.63.

Registry No. I, 7669-98-9; II, 3054-01-1; II undeuterated, 3054-01-1; IV, 57866-79-2; V, 83311-57-3; VI, 83311-58-4; VII, 83311-59-5; VIII, 83311-60-8; IX, 55559-18-7; X, 5908-08-7; XI, 83311-61-9; XII, 83311-62-0; XIII, 83311-63-1; XIV, 5907-95-9; XV, 83311-64-2; XVI, 83311-65-3; XVIII, 83311-67-5; XIX, 83333-36-2; Z-Gly-OH, 1138-80-3; H-L-Cys(Bzl)-ONp, 83311-68-6; Z-L-Cys(Bzl)-ONp, 3401-37-4; H-L-Cys(Bzl)-ONp·TFA, 83311-70-0; BOC-L-Cys(Bzl)-ONp, 3560-17-6; H-DL-Cys(Bzl)-ONp·HBr, 83311-71-1; H-Val-OMe, 4070-48-8; Z-Gly-OPcp, 4824-12-8; Z-Gly-L-Cys(Bzl)-OEt, 5854-92-2; *N*-acetyl-*S*-benzyl-DL-cysteine, 19538-71-7; *N*-carbobenzoxyglycyl-*S*-benzyl-DL-cysteine *p*-nitrophenyl ester, 83311-72-2; *N*-carbobenzoxyglycyl-*S*-benzyl-DL-cysteinyl-L-valine methyl ester, 83311-73-3; *N*-carbobenzoxyglycyl-*S*-benzyl-DL-(α-^{2}H)cysteine *p*-nitrophenyl ester, 83311-74-4; *N*-carbobenzoxy-*S*-benzyl-DL-cysteine *p*-nitrophenyl ester, 5619-11-4; *S*-benzyl-L-cysteine, 16741-80-3; deuterium, 7782-39-0.