(2.65 mmoles) of 0.5 *N* sodium ethoxide in ethanol. The mixture was shaken under a nitrogen atmosphere until a clear solution resulted (12 min). Work-up in the usual manner provided an amorphous powder which was crystallized from a small volume of methanol. The tripeptide XVI was obtained as 1.06 g (59.8%) of white needles, mp 131–132°, $[\alpha]^{24}D$ −9.5° (*c* 1.02, DMF).

Anal. Calcd for $C_{37}H_{39}N_3O_4S_2$: C, 64.79; H, 5.73; N, 6.13; S, 9.35. Found: C, 64.57; H, 5.62; N, 6.20; S, 9.22.

Attempted Detritylation of Ic Using *p*-Toluenesulfonic Acid.—When a solution of Ic was refluxed for 5 hr with 1 equiv of *p*-toluenesulfonic acid monohydrate in a benzene–ethanol mixture only starting material was obtained.

Attempted Detritylation of Ethyl N-Carbobenzoxy-S-triphenylmethyl-L-cysteinylglycinate.—Treatment of a chloroform solution of the protected dipeptide with a saturated solution of hydrogen chloride in chloroform for 80 min at room temperature provided only starting material, mp 112–113°.

Sulfur-Containing Polypeptides. III. S → N Benzoyl Group Migration in Cysteine Derivatives[1,2]

RICHARD G. HISKEY, TOMISHIGE MIZOGUCHI, AND TOSHISHIGE INUI

Venable Chemical Laboratory, The University of North Carolina, Chapel Hill, North Carolina

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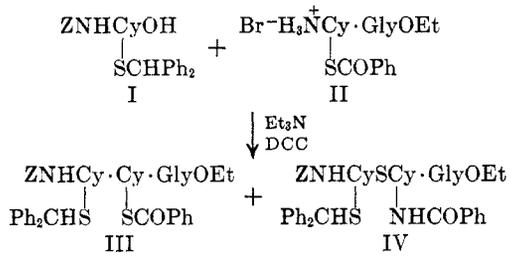
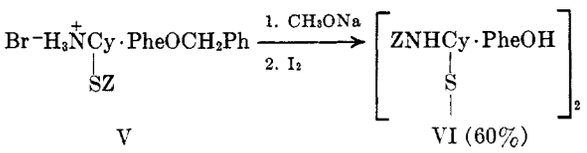
The S → N acyl migration of the S-benzoyl and S-carbobenzoxy groups has been studied. Migration of the benzoyl group occurs prior to coupling when N,N′-dicyclohexylcarbodiimide or *p*-nitrophenyl esters are employed. The direct coupling of methyl L-cysteinate with several N-protected amino acids has afforded the thiol peptide in good yields.

As part of an investigation concerned with the selective removal of various sulfur-protective groups from ethyl N-carbobenzoxy-L-cysteinyl-L-cysteinylglycinate, a sample of the protected tripeptide ester, III, was desired.[1] The synthetic procedure employed involved the coupling reaction between N-carbobenzoxy-S-diphenylmethyl-L-cysteine (I) and ethyl S-benzoyl-L-cysteinylglycinate (liberated by the action of triethylamine on the hydrobromide, II). Low yields (30%) of

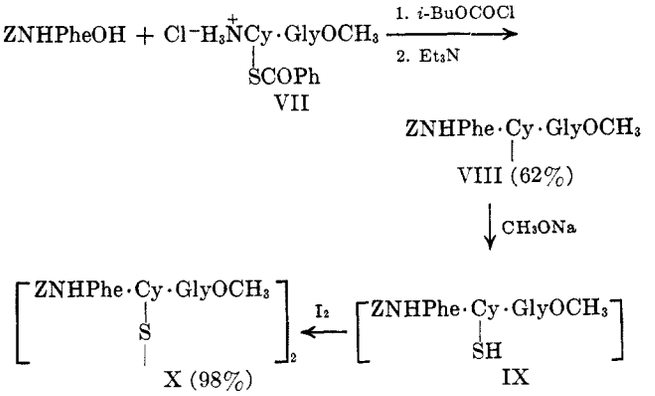


III were invariably obtained despite the fact that related derivatives were produced in reasonable yield. Since the substitution of II for other S-protected cysteine derivatives represented the major change in the reaction, the difficulty was believed to involve S → N benzoyl migration in the free base of II prior to addition to N,N′-dicyclohexylcarbodiimide (DCC).

The phenomenon of S → N acyl migration is well known and several detailed investigations have clarified the course of the reaction.[3,4] Patchornik, *et al.*,[5] have discussed the possibility of S → N carbobenzoxy group migration during peptide synthesis and have demonstrated that the dipeptide V is converted to the rearranged isomer VI when it was treated with strong base and the resulting thiol was oxidized with iodine. Despite these reports there appears to be no precedent for



S → N acyl migration during peptide-bond formation. On the contrary, Zervas, *et al.*,[6] have employed the S-benzoyl and S-carbobenzoxy groups to advantage in the synthesis of several cystine peptides and have apparently encountered no such difficulty. For example, when N-carbobenzoxy-L-phenylalanine was coupled[6] with methyl S-benzoyl-L-cysteinylglycinate (VII), the tripeptide VIII was obtained in 62% yield. Methanolysis of VIII and iodine oxidation of the resulting thiol, IX, provided the cystine peptide, X, in high yield. The present investigation was initiated in order to resolve the source of the low yields associated with the preparation of III and to define more fully any prob-



lems inherent in the use of the S-benzoyl and S-carbobenzoxy groups in the synthesis of cysteine peptides.

Initial efforts were directed toward the isolation and purification of the suspected thiol ester, ethyl N-benzoyl-S-(N-carbobenzoxy-S-diphenylmethyl-L-cysteinyl)-L-cysteinylglycinate (IV) from the DCC-coupling reaction of I and II. Although IV could not be obtained in a purified state from this reaction, the substance was isolated from the coupling of *p*-nitrophenyl

(1) Part II of this series: R. G. Hiskey, T. Mizoguchi, and H. Igeta, *J. Org. Chem.*, **31**, 1188 (1966).

(2) Supported by Grant A-3416 from the Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, U. S. Public Health Service.

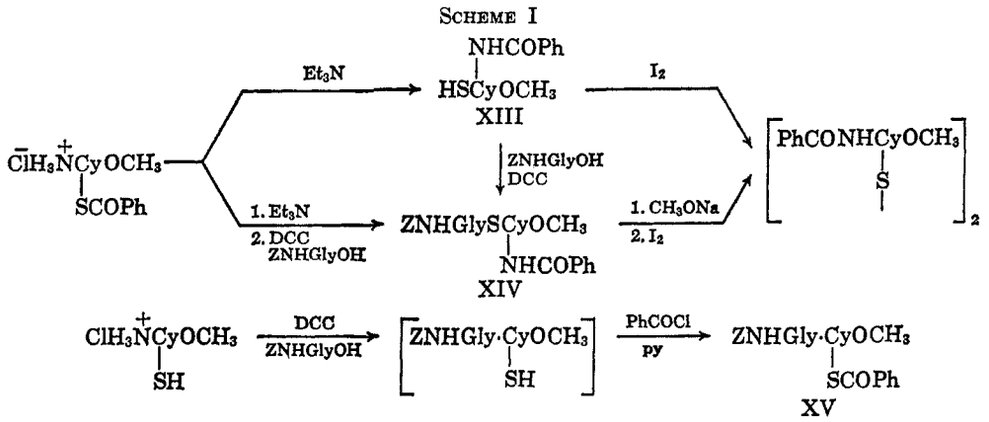
(3) R. B. Martin, R. I. Hedrick, and A. Parcell, *J. Org. Chem.*, **29**, 3197 (1964), and earlier references cited.

(4) H. S. Smith and G. Gorin, *ibid.*, **26**, 820 (1961).

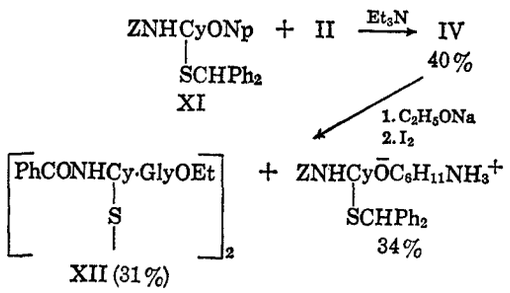
(5) M. Sokolovsky, M. Wilchek, and A. Patchornik, *J. Am. Chem. Soc.*, **86**, 1202 (1964).

(6) L. Zervas, I. Photaki, and N. Ghelis, *ibid.*, **85**, 1337 (1963).

SCHEME I



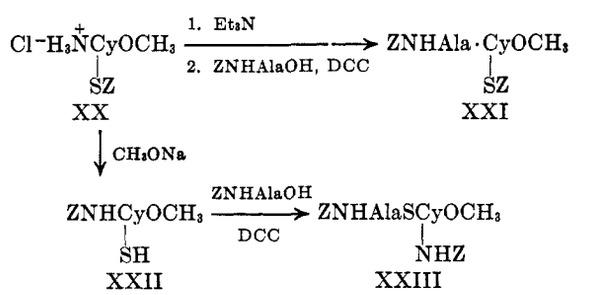
N-carbobenzoxy-S-diphenylmethyl-L-cysteinate (XI) and II. The thiol ester (IV) was characterized by



ethanolysis and oxidation to diethyl N,N′-dibenzoyl-L-cystinyldiglycinate (XII) and N-carbobenzoxy-S-diphenylmethyl-L-cysteine (I, isolated as the cyclohexylamine salt). Additional evidence supporting the possibility of S → N benzoyl migration was obtained by allowing II to react with 1 equiv of triethylamine; oxidation of the resulting thiol provided XII in 87% yield.

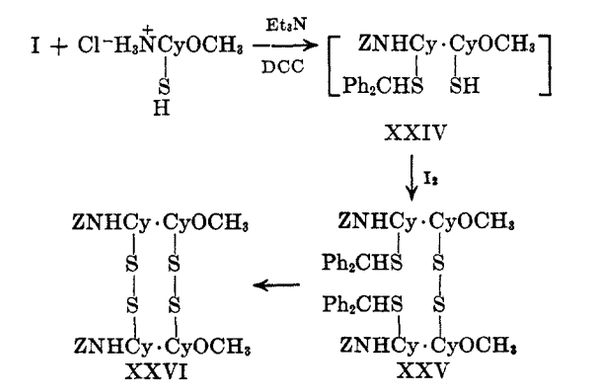
That rearrangement occurs prior to coupling with DCC was demonstrated by the following experiments (Scheme I). Methyl S-benzoyl-L-cysteinate hydrochloride was treated with 1 equiv of triethylamine and converted to the thiol XIII. Treatment of XIII with N-carbobenzoxyglycine and DCC provided methyl N-benzoyl-S-(N-carbobenzoxyglycyl)-L-cysteinate (XIV) in 82% yield. The same thiol ester was also obtained in 51% yield by the reaction of methyl S-benzoyl-L-cysteinate hydrochloride with 1 equiv of triethylamine in the presence of N-carbobenzoxyglycine and DCC. The authentic S-benzoylated dipeptide, methyl N-carbobenzoxyxglycyl-S-benzoyl-L-cysteinate (XV) was prepared by direct coupling of N-carbobenzoxyglycine with methyl L-cysteinate followed by benzoylation. The isomeric dipeptides and thiol esters, methyl N-benzoyl-S-(N-carbobenzoxy-L-phenylalanyl)-L-cysteinate (XVI), methyl N-carbobenzoxy-L-phenylalanyl-S-benzoyl-L-cysteinate (XVII), methyl N-benzoyl-S-(N-carbobenzoxy-L-alanyl)-L-cysteinate (XVIII), and methyl N-carbobenzoxy-L-alanyl-S-benzoyl-L-cysteinate (XIX), were prepared in a similar fashion. It is apparent from these experiments that S-benzoyl-L-cysteine derivatives rearrange rapidly, even in the presence of DCC or a *p*-nitrophenyl ester, to the corresponding N-benzoyl cysteines.

The S-carbobenzoxy derivative of methyl L-cysteinate hydrochloride (XX) was also treated with triethylamine in order to determine the extent of S → N acyl migration. In this experiment no rearrangement occurred. This result was verified by coupling XX with N-carbobenzoxy-L-alanine to yield methyl N-carbobenzoxy-L-alanyl-S-carbobenzoxy-L-cysteinate (XXI). The isomeric thiol ester (XXIII) was prepared from methyl N-carbobenzoxy-L-cysteinate (XXII). Thus, the S-carbobenzoxy group appears stable to the action of triethylamine but as previously reported[5]



migrates to nitrogen when sodium alkoxide is employed; XX was converted to XXII when treated with 2.5 equiv of sodium methoxide.

Although the tendency for S → N benzoyl migration limits the practical utility of the S-benzoyl protective group, the general concept of S → N acyl migration may prove of some value in the synthesis of cysteine peptides. Since the coupling of a cysteine ester with the appropriate N-protected amino acid provided the thiol (*e.g.*, the precursors to XV, XVII, and XIX) directly, the necessity for using a sulfur protective group could be eliminated. For example, dimethyl N,N′-bis-(N-carbobenzoxy-S-diphenylmethyl-L-cysteinyl)-L-cystinate (XXV) was desired as a precursor to XXVI. The substance was readily prepared by the reaction of I with methyl L-cysteinate hydrochloride using DCC and triethylamine or DCC in N,N-dimethylformamide.



Oxidation of the crude thiol (XXIV) provided XXV in 71% yield. Since similar thiols were prepared in lower over-all yield by the stepwise removal of S-protective groups,[1] this method probably represents the most advantageous route to molecules similar to XXV. Experiments pertaining to the conversion of XXV to XXVI will be reported separately.

Experimental Section[7]

Ethyl N-Benzoyl-S-(N-carbobenzoxy-S-diphenylmethyl-L-cysteinyl)-L-cysteinylglycinate (IV).—To a solution of 3.52 g (0.009 mole) of crude II in 35 ml of N,N-dimethylformamide was added 1.25 ml (0.009 mole) of triethylamine and 4.88 g (0.009 mole) of *p*-nitrophenyl N-carbobenzoxy-S-diphenylmethyl-L-cysteinate[6] (XI). The mixture was allowed to stand overnight at room temperature, diluted with ice–water, and extracted with ethyl acetate. The extract was washed with 1% potassium bicarbonate solution, dilute hydrochloric acid solution, and water. The dried extract was evaporated *in vacuo* to a yellow oil which crystallized when triturated with ether. The solid was recrystallized from a acetone–petroleum ether (bp 30–60°) mixture to yield 2.55 g (39.7%) of IV as needles, mp 137–138°, $[\alpha]^{23}D$ −91.9° (*c* 1.03, DMF).

Anal. Calcd for $C_{38}H_{39}N_3O_7S_2$: C, 63.93; H, 5.51; N, 5.89; S, 8.98. Found: C, 62.89, 62.94; H, 5.36, 5.49; N, 6.01; S, 8.94.

Ethanolysis of IV.—To a suspension of 1.43 g (2.0 mmoles) of IV in 42 ml of absolute ethanol was added 4.1 ml (2.05 mmoles) of 0.5 *N* sodium ethoxide in ethanol. The mixture was allowed to stand 12 min at room temperture and then treated with 2.5 ml of 1 *N* hydrochloric acid solution. The ethanol was removed *in vacuo;* the residue was dissolved in ethyl acetate and washed with saturated sodium chloride solution. The dried organic layer was evaporated to an oil, which exhibited two spots on tlc. The upper spot was nitroprusside negative; the lower spot, nitroprusside positive.

The oil was dissolved in 10 ml of ethanol and treated with 17.5 ml of 0.1 *N* iodine–potassium iodide in 90% ethanol solution. The ethanol was evaporated; the mixture was diluted with water and extracted with chlorform. The organic layer was washed with water, dried, and evaporated. Trituration of the residue with ether gave a crystalline solid which was recrystallized twice from ethanol to give 0.38 g (30.6%) of diethyl N,N′-bisbenzoyl-L-cystinyldiglycinate (XII), mp 210–211°, $[\alpha]^{24}D$ −194.9° (*c* 1.00, DMF).

Anal. Calcd for $C_{28}H_{34}N_4O_8S_2$: C, 54.35; H, 5.54; N, 9.06; S, 10.37. Found: C, 54.58; H, 5.69; N, 8.95; S, 9.84.

The ethereal wash solution was evaporated to a colorless oil. Hydrolysis of the oil with 5 ml of 1 *N* sodium hydroxide solution in 15 ml of methanol (2 hr, 25°), followed by acidification and treatment with cyclohexylamine, provided 0.35 g (34.3%) of N-carbobenzoxy-S-diphenylmethyl-L-cysteine cyclohexylamine salt, mp and mmp 144–145°, lit.[8] mp 142–143°.

S → N Benzoyl Migration in Ethyl S-Benzoyl-L-cysteinylglycinate Hydrobromide (II).—A solution of 0.84 g (2.14 mmoles) of crude II in 10 ml of chloroform was treated with 0.30 ml (2.14 mmoles) of triethylamine. The solution was allowed to stand overnight (thiol was detected in a few minutes) and the solvent was removed *in vacuo*. The residue was dissolved in ethyl acetate and washed with water, dilute hydrochloric acid solution, and water. The dried organic layer was evaporated *in vacuo* to a partially crystalline mass, which was dissolved in 30 ml of ethanol and oxidized with 0.1 *N* iodine–potassium iodide in 90% ethanol (16.5 ml). The reaction mixture was concentrated and diluted with water. The precipitated solid was collected and washed with cold water, a methanol–ether mixture (1:2), and absolute ether. The product, XII, 0.58 g (87.4%), was obtained as needles, mp 209–211°, mmp 210–211°, $[\alpha]^{24}D$ −194° (*c* 1.03, DMF).

S → N Benzoyl Migration in Methyl S-Benzoyl-L-cysteinate Hydrochloride.—A suspension of 0.566 g (2.06 mmoles) of methyl S-benzoyl cysteinate hydrochloride[6] in 10 ml of chloroform was treated with 0.30 ml (2.14 mmoles) of triethylamine. The reaction mixture was stirred 4 hr at room temperature and worked up in the usual manner to provide 0.40 g (82%) of methyl N-benzoyl-L-cysteinate (XIII), mp 81–82°.

A solution of 0.355 g (1.48 mmoles) of thiol in 10 ml of methanol consumed 14.0 ml of 0.1 *N* iodine–potassium iodide solution (94.3% of theoretical). The solvent was evaporated and the residue was dissolved in chloroform. The organic extract was washed with water, dilute sodium bicarbonate, and water and dried. Evaporation of the extract provided 0.31 g (88%) of dimethyl N,N′-bisbenzoyl-L-cystinate, mp 180–181°, $[\alpha]^{24}D$ −239.8° (*c* 0.95, DMF); lit. mp 176–178°,[9] 177–178°,[8] $[\alpha]^{25}D$ −233° (*c* 1, DMF).[6]

In another experiment the S → N benzoyl migration was followed by tlc. The rearrangement was complete in about 10 min.

Methyl N-Benzoyl-S-(N-carbobenzoxyglycyl)-L-cysteinate (XIV). A. From Methyl N-Benzoyl-L-cysteinate (XIII) and N-Carbobenzoxyglycine.—To a solution of 2.38 g (0.01 mole) of XIII in 15 ml of chloroform was added a solution of 2.09 g (0.01 mole) of N-carbobenzoxyglycine in 5 ml of chloroform and 10 ml of tetrahydrofuran; 2.06 g (0.01 mole) of DCC in 5 ml of chloroform was added immediately. The reaction mixture was stirred overnight at room temperature. The precipitated urea was filtered and the filtrate was worked up in the usual manner to provide 3.51 g (81.6%) of thiol ester, mp 154.9–156.5°. Recrystallization from ethyl acetate raised the melting point to 157–158°, $[\alpha]^{23.5}D$ −52.1° (*c* 1.0, MeOH).

Anal. Calcd for $C_{21}H_{22}N_2O_6S$: C, 58.59; H, 5.15; N, 6.51; S, 7.45. Found: C, 58.34; H, 5.16; N, 6.69; S, 7.24.

B. From Methyl S-Benzoyl-L-cysteinate Hydrochloride and N-Carbobenzoxyglycine.—To an ice-cold mixture of 16.5 g (0.06 mole) of methyl S-benzoyl-L-cysteinate hydrochloride and 8.4 ml (0.06 mole) of triethylamine in 100 ml of chloroform was added a solution of 12.5 g (0.06 mole) of N-carbobenzoxyglycine in 100 ml of chloroform and 80 ml of tetrahydrofuran; a solution of 12.3 g (0.06 mole) of DCC in 50 ml of chloroform was then added over 25 min. The reaction mixture was stirred overnight and worked up in the usual manner. The crude product, mp 152–154°, 13.2 g (51.4%), contained a minor amount of methyl N-carbobenzoxyglycyl-S-benzoyl-L-cysteinate (XV) as indicated by tlc. Recrystallization from ethyl acetate raised the melting point to 156.8–157.5°. A mixture melting point with the sample obtained as described in A was not depressed.

In another experiment methyl S-benzoyl-L-cysteinate hydrochloride was treated with separate solutions containing equivalent amounts of N-carbobenzoxyglycine triethylamine salt in chloroform and DCC in chloroform. The reaction was carried out in the usual way; tlc indicated the resulting product was a mixture of the S-benzoyl and N-benzoyl isomers (XIV and XV).

Methanolysis of XIV and Conversion to Dimethyl N,N′-Bisbenzoyl-L-cystinate.—To a suspension of 1.293 g (3.00 mmoles) of XIV in 24 ml of methanol was added 6.15 ml (3.07 mmoles) of 0.5 *N* sodium methoxide in methanol. The peptide dissolved within 10 min. The solution was acidified with 4.5 ml of glacial acetic acid and titrated with 0.1 *N* iodine–potassium iodide solution. The peptide consumed 26.2 ml (87% of theoretical) of the reagent. The solution was evaporated to dryness *in vacuo* and the residue was dissolved in chloroform. The organic extract was washed with water; the dried extract was evaporated to a semisolid. Trituration of the residue with ether provided 0.65 g (91%) of dimethyl N,N′-bisbenzoyl-L-cystinate, mp 172–174°. Recrystallization from methanol raised the melting point to 179–180°, $[\alpha]^{24}D$ −240.9° (*c* 1.13, DMF). A mixture melting point with an authentic sample was not depressed. A small amount of N-carbobenzoxyglycine was isolated from the mother liquor as the cyclohexylamine salt, mp 146–147°.

Methyl N-Carbobenzoxyglycyl-S-benzoyl-L-cysteinate (XV).—To a suspension of methyl L-cysteinate hydrochloride (1.71 g, 0.01 mole) in 15 ml of chloroform were added 1.40 ml (0.01 mole) of triethylamine and 2.09 g (0.01 mole) of N-carbobenzoxyglycine in 5 ml of chloroform and 10 ml of tetrahydrofuran. The resulting solution was treated with a solution containing 2.06 g (0.01

(7) Melting points are uncorrected. Elemental analyses were performed by Micro Tech Laboratories, Skokie, Ill. Optical rotations were taken using a Rudolph polarimeter Model 200 equipped with a Model 80 photoelectric attachment. Each purified substance exhibited a single spot on the silica gel G thin layer chromatogram. The solvent systems employed were chloroform–ethyl acetate (1:1) and benzene–dioxane–ethanol (12:12:1) unless otherwise noted.

(8) L. Zervas and I. Photaki, *J. Am. Chem. Soc.*, **84**, 3887 (1962).

(9) E. Fry, *J. Org. Chem.*, **15**, 438 (1950).

mole) of DCC in 5 ml of chloroform. The reaction mixture was stirred overnight at room temperature. The usual work-up provided 3.0 g of crude peptide ester as a syrup; the material gave a positive nitroprusside test.

The syrup was dissolved in 5 ml of pyridine and treated with 1.20 ml (0.01 mole) of benzoyl chloride. The solution was stirred at 0° for 1 hr and poured on ice, and the oil was extracted with ethyl acetate. The extract was washed with dilute hydrochloric acid, dilute sodium bicarbonate, and water. The dried extract was evaporated to a yellow oil (3.94 g). The addition of ether provided 2.54 g (59%) of XV, mp 101–101.5°, $[\alpha]^{23.5}D$ −7.2° (*c* 1.2, MeOH), from benzene–petroleum ether.

Anal. Calcd for $C_{21}H_{22}N_2O_6S$: C, 58.59; H, 5.15; N, 6.51; S, 7.45. Found: C, 58.32; H, 5.17; N, 6.45; S, 7.67.

Methyl N-Carbobenzoxy-L-phenylalanyl-S-benzoyl-L-cysteinate (XVII).—A solution containing 2.40 g (8.0 mmoles) of N-carbobenzoxy-L-phenylalanine,[10] 1.37 g (8.0 mmoles) of methyl L-cysteinate hydrochloride, and 1.12 ml (8.0 mmoles) of triethylamine in 30 ml of chloroform was treated with 1.65 g (8.0 mmoles) of DCC in 5 ml of chloroform. The crystalline crude thiol (1.64 g), obtained by the usual work-up, melted at 125–127°.

The crude thiol was dissolved in 6 ml of pyridine and treated with 0.70 ml (6.1 mmoles) of benzoyl chloride at 0°. After 1 hr the reaction mixture was worked up as previously described. The S-benzoate was obtained as 1.38 g (34%) of crystalline solid, mp 130–132°. Recrystallization from methanol raised the melting point to 139–140°, $[\alpha]^{23}D$ −38.5° (*c* 1.2, MeOH).

Anal. Calcd for $C_{28}H_{28}N_2O_6S$: C, 64.60; H, 5.42; N, 5.38; S, 6.16. Found: C, 64.70; H, 5.46; N, 5.60; S, 6.16.

Methyl N-Benzoyl-S-(N-carbobenzoxy-L-phenylalanyl)-L-cysteinate (XIV).—To a solution of 1.93 g (8.1 mmoles) of methyl N-benzoyl-L-cysteinate (XIII) in 15 ml of chloroform was added a solution of 2.42 g (8.1 mmoles) of N-carbobenzoxy-L-phenylalanine in 15 ml of chloroform. The solution was treated with 1.67 g (8.1 mmoles) of DCC in 5 ml of chloroform and allowed to stand at room temperature overnight. The usual work-up provided 2.70 g (64%) of thiol ester XVI, mp 128–130°. Recrystallization from methanol raised the melting point to 132.5–133°, $[\alpha]^{23}D$ −91.8° (*c* 1.0, MeOH).

Anal. Calcd for $C_{28}H_{28}N_2O_6S$: C, 64.60; H, 5.42; N, 5.38; S, 6.16. Found: C, 64.33; H, 5.69; N, 5.49; S, 6.23.

A mixture with the S-benzoyl peptide (XVII) melted at 123–128.5°. The methanolysis of XVI and the conversion to dimethyl N,N′-bisbenzoyl-L-cystinate was carried out in the manner described for XIV. Iodine titration revealed 87.5% of thiol; the yield of dimethyl N,N′-bisbenzoyl-L-cystinate was 63%, mp 178.5–179.5°. A mixture melting point with an authentic sample was not depressed.

Methyl N-Carbobenzoxy-L-alanyl-S-benzoyl-L-cysteinate (XIX).—The coupling of methyl L-cysteinate hydrochloride (1.37 g, 8.1 mmoles) and 1.78 g (8.0 mmoles) of N-carbobenzoxy-L-alanine with 1.12 ml (8.0 mmoles) of triethylamine and 1.65 g (8.0 mmoles) of DCC was carried out in the usual manner in 35 ml of chloroform. The crude thiol (1.90 g) melted at 114–116°.

The thiol was benzoylated with 0.85 ml (7.4 mmoles) of benzoyl chloride in 5 ml of pyridine. The S-benzoyl peptide (XIX), 1.72 g (48.6%), melted at 129–131°. Recrystallization from methanol raised the melting point to 135–135.5°, $[\alpha]^{24}D$ −32.1° (*c* 1.0, MeOH).

Anal. Calcd for $C_{22}H_{24}N_2O_6S$: C, 59.44; H, 5.44; N, 6.30; S, 7.22. Found: C, 59.32; H, 5.39; N, 6.47; S, 7.09.

Methyl N-Benzoyl-S-(N-carbobenzoxy-L-alanyl)-L-cysteinate (XVIII).—A solution of 1.88 g (7.9 mmoles) of methyl N-benzoyl-L-cysteinate (XIII) and 1.76 g (7.9 mmoles) of N-carbobenzoxy-L-alanine in 30 ml of chloroform was treated with 1.63 g (7.9 mmoles) of DCC in 5 ml of chloroform and allowed to stand at room temperature overnight. The usual work-up provided 2.80 g (81%) of thiol ester XVIII, mp 108–112°. Although several solvent systems were employed for recrystallization, XVIII was usually obtained as a gelatinous powder which melted at 115–120°. The substance exhibited a single spot on tlc; the R_f value (0.60) was different from that of XIX (0.47).

The methanolysis of XVIII and the conversion to dimethyl N-N′-bisbenzoyl-L-cystinate was carried out in the same manner as described for XIV. Iodine titration revealed 82.0% of thiol; the yield of dimethyl N-N′-bisbenzoyl-L-cystinate was 58%, mp 178–179°. A mixture melting point with an authentic sample was not depressed.

S → N Carbobenzoxy Group Migrations in Methyl S-Carbobenzoxy-L-cysteinate (XX). A. Using 1 Equiv of Triethylamine.—To a suspension of 0.612 g (2.0 mmoles) of XX in 15 ml of chloroform was added 0.28 ml (2.0 mmoles) of triethylamine. The mixture was stored at room temperature for 3 hr and poured onto water and the chloroform layer separated. The organic layer was washed with 1 *N* hydrochloric acid, dilute sodium bicarbonate, and water. Evaporation of the dried extract provided no residue.

B. Using 2.5 Equiv of Sodium Methoxide.—To a solution of 0.61 g (2.0 mmoles) of XX in 10 ml of methanol was added 10 ml (5 mmoles) of 0.5 *N* sodium methoxide in methanol solution. The solution was kept at room temperature for 10 min, acidified with 1 *N* hydrochloric acid, and titrated with 0.1 *N* iodine–potassium iodide solution. The thiol consumed 15.0 ml (75% of theoretical) of iodine. The solvent was removed and the residue was extracted with ethyl acetate. The extract was washed with dilute sodium thiosulfate solution and water. The dried extract was evaporated to dryness and the resulting oil was subjected to tlc. The chromatogram exhibited a single spot identical with that of an authentic dimethyl N,N′-biscarbobenzoxy-L-cystinate.

Methyl N-Carbobenzoxy-L-alanyl-S-carbobenzoxy-L-cysteinate (XXI).—To a suspension of 1.83 g (6.0 mmoles) of methyl S-carbobenzoxy-L-cysteinate hydrochloride in 15 ml of chloroform were added 0.84 ml (6.0 mmoles) of triethylamine and a solution containing 1.34 g (6.0 mmoles) of N-carbobenzoxy-L-alanine in 15 ml of chloroform. A solution of 1.23 g (6.0 mmoles) of DCC in 5 ml of chloroform was added and the reaction mixture was allowed to stand overnight. The usual work-up provided 2.07 g (87.4%) of XXI, mp 95–96°. Recrystallization from ethyl acetate–petroleum ether (bp 60–90°) raised the melting point to 95.8–96.2°, $[\alpha]^{24.5}D$ −37.4° (*c* 1.14, MeOH).

Anal. Calcd for $C_{23}H_{26}N_2O_7S$: C, 58.21; H, 5.52; N, 5.90; S, 6.78. Found: C, 58.36; H, 5.62; N, 5.99; S, 6.72.

Methyl N-Carbobenzoxy-S-(N-carbobenzoxy-L-alanyl)-L-cysteinate (XXIII).—To a mixture of 2.0 g (7.4 mmoles) of XXII and 1.6 g (7.2 mmoles) of N-carbobenzoxy-L-alanine in 30 ml of chloroform was added a solution of 1.5 g (7.3 mmoles) of DCC in 5 ml of chloroform. After stirring 4.5 hr at room temperature, the mixture was worked up in the usual manner. The resulting oil crystallized on standing overnight to yield 2.85 g (83.9%) of product, mp 85–88.5°. One recrystallization from ethyl acetate–petroleum ether (bp 30–60°) raised the melting point to 90–91.5°, $[\alpha]^{24.5}D$ −42.3° (*c* 1.07, MeOH). A mixture with N,S-dicarbobenzoxy ester XXI melted at 79.0–86.5°.

Anal. Calcd for $C_{23}H_{26}N_2O_7S$: C, 58.21; H, 5.52; N, 5.90; S, 6.78. Found: C, 58.25; H, 5.47; N, 6.02; S, 6.82.

Dimethyl N,N′-Bis(N-carbobenzoxy-S-diphenylmethyl-L-cysteinyl)-L-cystinate (XXV). A. Using Triethylamine.—To a suspension of 0.858 g (0.5 mmole) of methyl L-cysteinate hydrochloride in 21 ml of methylene chloride was added 0.70 ml (0.5 mmole) of triethylamine. The mixture was treated with 2.11 g (0.5 mmole) of I[8] (liberated from the cyclohexylamine salt) and a solution containing 1.07 g (0.52 mmole) of DCC in 10 ml of methylene chloride. The addition required 30 min; the mixture was stored overnight at room temperature. Work-up in the usual manner provided a thick oil, which was dissolved in ether, filtered, and evaporated. The residue was dissolved in 25 ml of ethanol and oxidized with 0.1 *N* iodine–potassium iodide reagent. Evaporation of reaction mixture provided 1.90 g (70.6%) of XXV as fine needles, mp 154–156°. Recrystallization from ethanol raised the melting point to 155–156°, $[\alpha]^{23}D$ −86.8° (*c* 1.02, DMF).

Anal. Calcd for $C_{56}H_{52}N_4O_{10}S_4$: C, 62.55; H, 5.44; N, 5.21; S, 11.93. Found: C, 62.35; H, 5.25; N, 5.24; S, 12.06.

B. Coupling in the Absence of Triethylamine.—A cold solution containing 1.405 g (3.3 mmoles) of I and 0.574 g (3.3 mmoles) of methyl L-cysteinate hydrochloride, in 10 ml of N,N-dimethylformamide, was treated with 0.687 g (3.3 mmoles) of DCC. The mixture was allowed to stand at room temperature for 5 hr and filtered to remove N,N′-dicyclohexylurea (0.60 g, 80.4%). The filtrate was treated with 0.5 ml (3.6 mmoles) of triethylamine and the solution was allowed to stand overnight. The mixture was diluted with cold water and extracted with ethyl acetate and the extract was worked up in the usual manner. Oxidation as previously described provided 0.46 g (25.7%) of XXV, mp 154–156°, mmp 155–156°, $[\alpha]^{23}D$ −86.2° (*c* 1.125, DMF).

(10) W. Grassmann and E. Wunsch, *Chem. Ber.*, **91**, 462 (1958).