previous phenacylide. Upon recrystallization of the solid as before a 95% yield of product was obtained: mp 56–57°.

C. Silver Oxide Method.—The procedure followed was that of Bohme and Krause.[12] To a suspension of silver oxide (11.58 g, 0.05 mole) in 150 ml of water was added dropwise a solution of dimethyl phenacyl sulfonium bromide (13.06 g, 0.05 mole) in 400 ml of water. Precipitation occurred and, after addition was completed, the solution was filtered and the water was removed by evaporation on a rotary evaporator. The black, syrupy material was dissolved in chloroform and filtered, and the chloroform was evaporated. The residue was kept in a desiccator over sulfuric acid. Crystals were formed and were separated by filtration (7.5 g, 83%), mp 50–55°. The infrared spectrum was identical with that from above methods.

Repeated recrystallization of the sample from benzene–petroleum ether gave a pure sample which was dried over phosphorus pentoxide *in vacuo*. *Anal.* Calcd for $C_{10}H_{12}OS$:[24] C, 66.62; H, 6.76; S, 17.79. Found: C, 66.35; H, 6.91; S, 17.53.

(24) Although molecular weight determinations were always high (Calcd: 180. Found: 244) for this compound, the 4-nitro substituted ylid V, R = CH_3; R′ = 4-$C_5H_4NO_2$, yielded a correct molecular weight by osmometric techniques (Calcd: 224. Found: 227.).

The remaining ylids in Table I were recrystallized from benzene. Repeated efforts to crystallize the ester and amide were unsuccessful.

A solution of the ylid (0.20 g) in ether–chloroform was treated with anhydrous hydrogen bromide. Dimethyl phenacyl sulfonium bromide, 0.26 g (90%), mp 137–138°, was precipitated.

Hydrolysis of (Dimethylsulfuranylidene)acetophenone.—Seven grams (0.039 mole) of the above ylid was dissolved in aqueous potassium hydroxide (70 ml, 1 *N*) and the mixture was refluxed 19 hr. The yellow solution was cooled and extracted with ether (two 10-ml portions), then methylene chloride (two 10-ml portions). The aqueous layer was acidified with 10% hydrochloric acid and extracted with methylene chloride (two 20-ml portions), and the organic layer was dried over magnesium sulfate. Concentration of this solution gave 4.3 g (91%) of benzoic acid.

Measurement of p*K* Values (Figure 1).—The p*K* values were determined by the method given in ref 3.

Sulfur-Containing Polypeptides. II. Selective Removal of S-Protective Groups from Some L-Cysteinyl-L-cysteine Derivatives[1,2]

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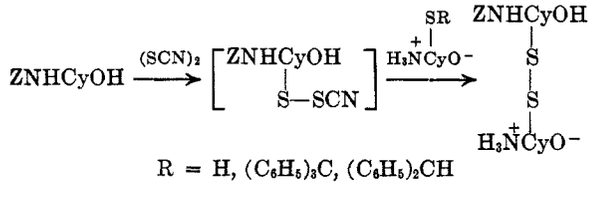
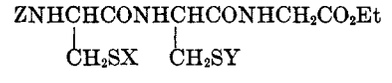
The synthesis of four derivatives of ethyl N-carbobenzoxy-L-cysteinyl-L-cysteinylglycinate is described. The S-protective groups studied were S-triphenylmethyl, S-diphenylmethyl, and S-benzoyl. Mercury(II) acetate was found to cleave selectively the S-triphenylmethyl group while sodium ethoxide selectively removed the S-benzoyl group.

An important aspect of the synthetic problem associated with the preparation of polypeptides "cross-lined" or "looped" at known positions by cysteine residues is the stepwise formation of the disulfide bonds. This, in turn, requires the selective removal of various sulfur-protective groups from the appropriate cysteine residues. One approach to this situation has involved the use of S-protective groups which would exhibit a reactivity gradient toward thiocyanogen, a reagent found[3,4] to convert various cysteine derivatives to the corresponding sulfenylthiocyanates; the latter behave similarly to thiocyanogen and provide unsymmetrical cysteine derivatives when allowed to react with another cysteine compound. In order to obtain maximum



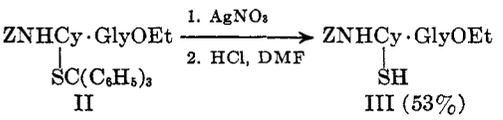
R = H, $(C_6H_5)_3C$, $(C_6H_5)_2CH$

utility from the sulfenylthiocyanate method of disulfide synthesis it appears to be necessary to employ the thiol group of cysteine as well as the S-tri- and -diphenylmethyl thioether derivatives. Therefore, synthetic methods for the conversion of specific S-blocked cysteine residues to the free thiol are required. Although several recent reports[5] have concerned methods for the removal of a single S-protective group in various cysteine peptides, no data are available on the removal of one S-blocking group in the presence of another.[6] The present report concerns the selective cleavage of several S-protective groups (X and Y) in the model tripeptide, ethyl N-carbobenzoxy-L-cysteinyl-L-cysteinylglycinate (I).



Ia, X = $(C_6H_5)_2CH$; Y = $(C_6H_5)_3C$
b, X = $(C_6H_5)_3C$; Y = $(C_6H_5)_2CH$
c, X = C_6H_5CO; Y = $(C_6H_5)_3C$
d, X = $(C_6H_5)_2CH$; Y = C_6H_5CO

The S-triphenylmethyl group was initially reported[7] to be cleaved by the action of hydrogen chloride in chloroform. Subsequently Zervas and Photaki[5a] demonstrated that ethyl N-carbobenzoxy-S-triphenylmethyl-L-cysteinylglycinate (II) was converted to the cysteine derivative (III) by the action of silver nitrate



(1) Part I of this series: R. G. Hiskey and J. B. Adams, Jr., *J. Am. Chem. Soc.*, **87**, 3969 (1965).

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

(3) R. G. Hiskey and W. P. Tucker, *J. Am. Chem. Soc.*, **84**, 4796 (1962).

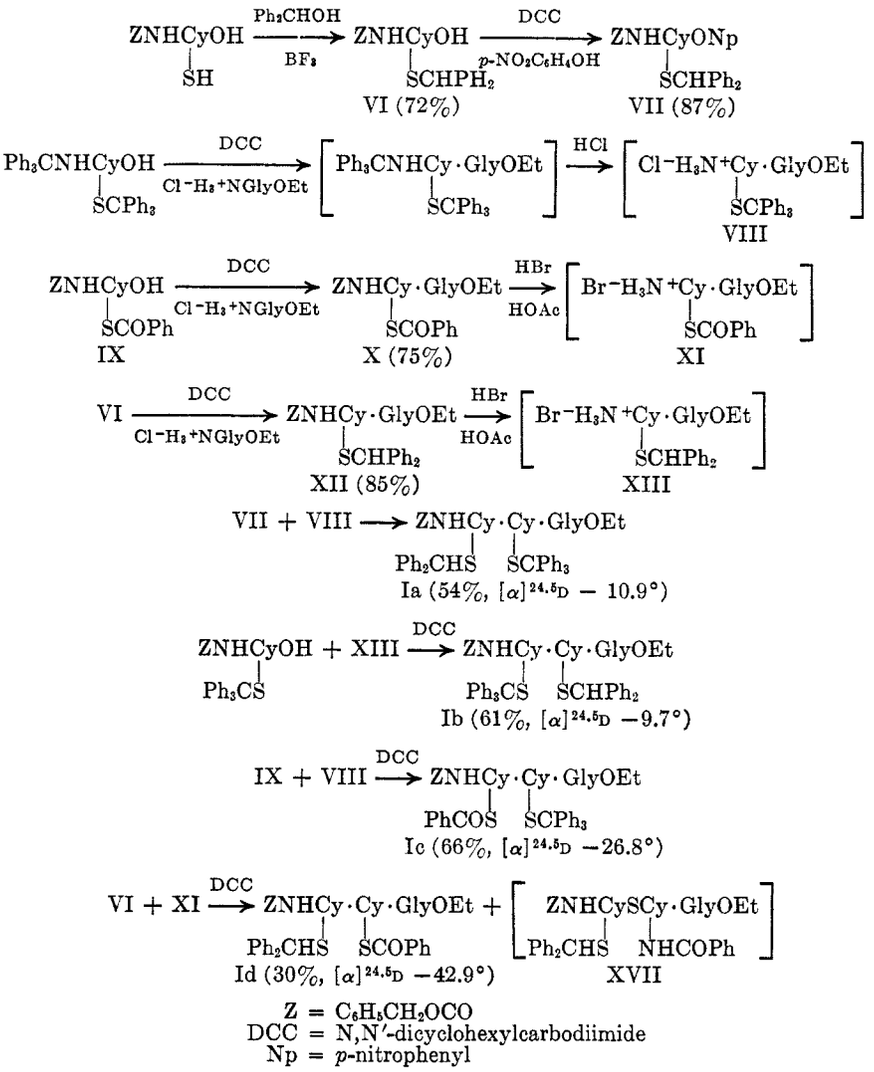
(4) R. G. Hiskey and D. N. Harpp, *ibid.*, **87**, 3965 (1965).

(5) (a) L. Zervas and I. Photaki, *ibid*, **84**, 3887 (1962); (b) L. Zervas, I. Photaki, and N. Ghelis, *ibid.*, **85**, 1337 (1963); (c) M. Sokolovsky, M. Wilchek, and A. Patchornik, *ibid.*, **86**, 1202 (1964).

(6) A referee has informed us that a paper on this topic by L. Zervas, *et al.*, has appeared: *ibid.*, **87**, 4922 (1965).

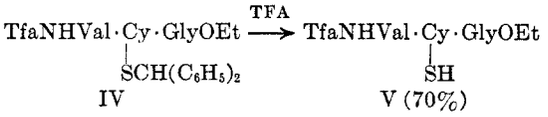
(7) L. Velluz, G. Amiard, J. Bartos, B. Goffinet, and R. Heymes, *Bull. Soc. Chim. France*, 1464 (1956).

CHART I
SYNTHESIS OF TRIPEPTIDES Ia–d



Z = $C_6H_5CH_2OCO$
DCC = N,N′-dicyclohexylcarbodiimide
Np = *p*-nitrophenyl

in a pyridine–methanol solvent. Recently, however, Carroll, *et al.*,[8] have reported that the removal of the S-triphenylmethyl group from several N-substituted S-triphenylmethylcysteamines could only be accomplished using mercury(II) acetate in ethanol, followed by treatment with hydrogen sulfide. The desired cysteamines could not be obtained by use of the reagents previously reported.[5a,7] Another protective group desired for study was the S-diphenylmethyl group. Acid hydrolysis experiments[5a] have indicated the S-diphenylmethyl group is considerably more stable than S-triphenylmethyl thioethers. The former has been cleaved by refluxing trifluoroacetic acid[5a] (IV → V); both groups are removed by warm hydrogen bromide in refluxing acetic acid.[5a]



In addition to acid-labile S-protective groups, significant studies on alkali-labile thiol esters have appeared. The S-benzoyl[5b] and S-carbobenzoxy[5c] derivatives have been found to be stable to acid but readily removed with sodium alkoxides. Thus, both acid- and alkali-labile groups were available and could presumably be incorporated into the tripeptide I. The synthetic routes to the desired derivatives (Ia–d) are outlined in Chart I.

The synthesis of Ia and Ic involved the coupling of ethyl S-triphenylmethyl-L-cysteinylglycinate (VIII) with the appropriate N,S-protected cysteine (VII or IX). The synthetic value of this route is considerably diminished by the noncrystalline nature of VIII and its precursors. However, since N,S-ditriphenylmethyl-L-cysteine is one of the few derivatives of S-triphenylmethyl-L-cysteine which can be coupled with a carboxy-protected amino acid and then deblocked at the amino function, this sequence was the procedure of choice. The preparation of Ib and Id (*via* XI and XIII) presented no difficulties of this type since both the S-benzoyl and S-diphenylmethyl groups are stable to hydrogen bromide in acetic acid at room temperature; thus the crystalline N-carbobenzoxy derivatives could be utilized.

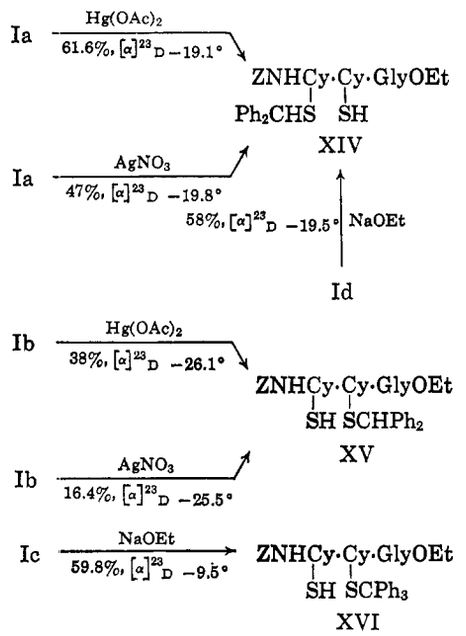
The coupling steps to provide the desired tripeptides, Ia–c, proceeded in reasonable yields; however, in the case of Id (obtained from VI and XI) only 30% of the tripeptide could be isolated. The low yield of Id

(8) F. I. Carroll, H. M. Dickson, and M. E. Wall, *J. Org. Chem.*, **30**, 33 (1965). E. Galantay, H. Engel, A. Szabo, and J. Fried [*ibid.*, **29**, 3560 (1964)] have utilized mercury(II) chloride in 1,2-dimethoxyethane.

was ultimately traced to S → N benzoyl migration in XI prior to coupling. Therefore a substantial amount of the reaction product appeared as the thiol ester, XVII. The experiments which establish this reaction course and other reactions exploiting this property of S-benzoylcysteinyl peptides are described in the accompanying report.[9]

With the desired tripeptides available various methods for the selective removal of the incorporated S-protective groups were studied. The results of these experiments are outlined in Chart II. Deblocking of

CHART II
SELECTIVE REMOVAL OF S-PROTECTIVE GROUPS FROM TRIPEPTIDES Ia–Id



either Ia or Id provided ethyl N-carbobenzoxy-S-diphenylmethyl-L-cysteinyl-L-cysteinylglycinate (XIV) in 47–62% yield. The superiority of mercury(II) acetate for S-triphenylmethyl group removal is evident from these experiments. Since the optical purity of Ia and Id has not been established, a firm conclusion cannot be reached regarding the amount of racemization involved in the S-deblocking step. However, the fact that the optical rotations of XIV obtained from either source are similar suggests that the S-triphenylmethyl and S-benzoyl groups can be removed without racemization of a neighboring protected cysteine residue. Hydrolysis of Ib provided ethyl N-carbobenzoxy-L-cysteinyl-S-diphenylmethyl-L-cysteinylglycinate (XV) but in much lower yield than XIV. Mercury(II) acetate was again the more effective catalyst. The infrared spectra of the isomeric thiols (XIV and XV) as well as those of the precursors (Ia and Ib) were essentially identical; this similarity is also reflected in the R_f values on thin layer chromatography (tlc). The fact that both materials exhibited thiol absorption at 2610 cm^{-1} and gave a negative test with ninhydrin but the characteristic color with the sodium nitroprusside reagent indicates that no N → S acyl migration occurred during the cleavage. These data clearly indicate that both the S-triphenylmethyl and S-benzoyl can be selectively removed in the presence of the S-diphenylmethyl group, although the actual yield of thiol was somewhat lower than anticipated.

Attempts to remove the S-triphenylmethyl group from Ia and b with various acids were less successful. Treatment of either Ia or Ib with hydrogen chloride in chloroform provided only recovered starting material. Treatment of Ia with dry hydrogen chloride in acetic acid or with boron trifluoride in 98% formic acid produced extensive decomposition. The use of aqueous hydrochloric acid in acetic acid, however, afforded essentially pure thiol and may represent a useful procedure for S-triphenylmethyl hydrolysis.

Ethanolysis of Ic with sodium ethoxide illustrates the compatibility of the S-triphenylmethyl and S-benzoyl groups. The product, ethyl N-carbobenzoxy-L-cysteinyl-S-triphenylmethyl-L-cysteinylglycinate (XVI) was obtained in 60% yield. Attempted cleavage of Ic with *p*-toluenesulfonic acid or with diethylamine provided only recovered starting material.

Experimental Section[10]

***p*-Nitrophenyl N-Carbobenzoxy-S-diphenylmethyl-L-cysteinate (VII).**—A suspension of 6.5 g (0.016 mole) of N-carbobenzoxy-S-diphenylmethyl-L-cysteine cyclohexylamine salt[11] (VI) in 150 ml of ethyl acetate was shaken with two 50-ml portions of 0.5 *N* hydrochloric acid. The organic layer was washed with saturated sodium chloride solution, dried, and evaporated *in vacuo* to yield 5.04 g (95.7%) of the crystalline acid.

The acid was dissolved in 27 ml of ethyl acetate and treated with 1.8 g (0.013 mole) of *p*-nitrophenol and 3.0 g (0.014 mole) of DCC. The mixture was stored overnight at room temperature, filtered, washed with 5% sodium bicarbonate solution and saturated sodium chloride solution, and dried. Evaporation of the organic layer provided a yellow oil which crystallized when triturated with absolute ethanol. Recrystallization from absolute ethanol provided 5.52 g (84.9%) of fine needles, mp 93–96°. The analytical sample, recrystallized twice from absolute ethanol, melted at 96–98°, $[\alpha]^{22}D$ −33.7° (*c* 1.19, DMF).

Anal. Calcd for $C_{30}H_{26}N_2O_6S$: C, 66.40; H, 4.83; N, 5.16; S, 5.91. Found: C, 66.79; H, 4.98; N, 5.30; S, 5.94.

Ethyl N-Triphenylmethyl-L-cysteinylglycinate Hydrochloride (VIII).—VIII was prepared according to the procedure of Amiard, *et al.*[12] The pale yellow powder was coupled directly without further purification: R_f (tlc) 0.64, 95% ethanol (ninhydrin), single spot.

Ethyl N-Carbobenzoxy-S-diphenylmethyl-L-cysteinyl-S-triphenylmethyl-L-cysteinylglycinate (Ia).—To a solution containing 10.7 g (0.022 mole) of crude VIII in 86 ml of DMF was added 3.06 ml (0.022 mole) of triethylamine. The mixture was stirred for several minutes and treated with 10.7 g (0.0197 mole) of VII. The reaction mixture was stirred overnight at room temperature, poured onto a 5% sodium bicarbonate–ice mixture, and filtered. The precipitated tripeptide was washed with 5% sodium bicarbonate, 1 *N* hydrochloric acid solution, and water. The solid was then dried over calcium chloride and washed once with acetone. Recrystallization from ethyl acetate provided 10.2 g (54.3%) of Ia, mp 192–194°, $[\alpha]^{24.5}D$ −10.9° (*c* 1, DMF).

Anal. Calcd for $C_{50}H_{49}N_3O_6S_2$: C, 70.41; H, 5.79; N, 4.94; S, 7.52. Found: C, 70.61; H, 5.90; N, 4.92; S, 7.55.

Ethyl N-Carbobenzoxy-S-benzoyl-L-cysteinylglycinate (X).—X was prepared according to the procedure of Zervas, *et al.*,[5b] in 70.7% yield, mp 154–156° (lit.[5b] mp 153°).

(9) R. G. Hiskey, T. Mizoguchi, and T. Inui, *J. Org. Chem.*, **31**, 1192 (1966).

(10) Melting points are uncorrected. Elemental analyses were performed by Micro Tech Laboratories, Skokie, Ill. Optical rotations were taken using a Rudolph Polarimeter with a photoelectric attachment. Each purified substance described 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.

(11) L. Zervas and I. Photaki, *J. Am. Chem. Soc.*, **84**, 3893 (1962).

(12) G. Amiard, R. Heymes, and L. Velluz, *Bull. Soc. Chim. France*, 698 (1956).

Ethyl N-Carbobenzoxy-S-diphenylmethyl-L-cysteinyl-S-benzoyl-L-cysteinylglycinate (Id).—A suspension of 2.30 g (5.18 mmoles) of X in 30 g of 12% (w/w) hydrogen bromide in acetic acid was allowed to stand at room temperature for 1 hr. The solvent was removed *in vacuo* and the residue was washed three times with 100-ml portions of dry ether. The resulting oil was dried over calcium chloride and potassium hydroxide to provide 1.87 g of crude ethyl S-benzoyl-L-cysteinylglycinate hydrobromide (XI).

A cold solution containing 1.66 g (4.25 mmoles) of XI in 22 ml of methylene chloride was treated with 0.59 ml of triethylamine followed by 1.79 g (4.25 mmoles) of VI (liberated from the cyclohexylamine salt as previously described). The resulting solution was treated with 0.95 g (4.6 mmoles) of DCC and the reaction mixture was stirred at 10° for 5 hr. The solution was filtered and evaporated *in vacuo*, and the residue was dissolved in ethyl acetate. The organic extract was washed successively with dilute potassium bicarbonate solution, dilute hydrochloric acid solution, and water and dried. Evaporation of the solvent provided a crystalline solid which was washed with 20 ml of dry ether. The solid was dissolved in a minimum volume of tetrahydrofuran and cooled. The precipitated N,N'-dicyclohexylurea was filtered and the filtrate was evaporated. The residue was recrystallized from ethanol to provide 0.99 g (30%) of Id, mp 152–153°, $[\alpha]^{23}D$ −42.9° (*c* 1.04, 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, 64.06; H, 5.59; N, 5.93; S, 9.00.

Ethyl N-Carbobenzoxy-S-diphenylmethyl-L-cysteinyl-L-cysteinylglycinate (XIV). A. *Via* Mercury(II) Acetate.—To a warm solution of 6.0 g (7.05 mmoles) of Ia in 600 ml of an absolute ethanol–ethyl acetate (1:1) mixture was added a solution containing 2.94 g (9.3 mmoles) of mercury(II) acetate in 100 ml of absolute ethanol. The solution was heated under gentle reflux for 3 hr and cooled overnight. The solvent was removed *in vacuo* and the residue was washed with two 25-ml portions of cold methanol. The yellow powder was suspended in 100 ml of ethyl acetate and treated with excess hydrogen sulfide. Charcoal was added to the mixture and the solid material was filtered. Evaporation of the filtrate afforded a white residue, XIV, which crystallized as needles from methanol, 2.65 g (61.6%), mp 162–163°. The substance gave a positive sodium nitroprusside test and exhibited an infrared absorption peak at 2610 cm^{-1} (KBr). The analytical sample was recrystallized twice from methanol and had mp 165–166°, $[\alpha]^{23}D$ −19.1° (*c* 1.04, DMF).

Anal. Calcd for $C_{31}H_{35}N_3O_6S_2$: C, 61.05; H, 5.78; N, 6.89; S, 10.50. Found: C, 61.28; H, 6.33; N, 7.02; S, 10.12.

B. *Via* Silver Nitrate.—A warm solution containing 1.70 g (2.0 mmoles) of Ia in 75 ml of an ethyl acetate–ethanol (2:1) mixture was treated with 0.34 g (2.0 mmoles) of silver nitrate in 15 ml of absolute ethanol containing 0.16 ml (2.0 mmoles) of pyridine. The mixture was heated for 1.5 hr on the steam bath. The precipitate was collected and washed with 25 ml of cold absolute ethanol and 25 ml of dry ether to yield 1.31 g (91.5%) of the silver mercaptide of XIV. The mercaptide was suspended in 50 ml of ethyl acetate and treated with hydrogen sulfide as previously described. Crystallization of XIV from methanol provided 0.85 g (70%) of white needles, mp 154–156°. Elution of the material (chloroform–ethyl acetate, 95:5) from a silicic acid column (recovery 67%, yield of pure product 47%) provided the purified peptide, mp 165–166°, $[\alpha]^{23}D$ −19.8° (*c* 1.01, DMF). A mixture melting point with the sample of XIV prepared as described in A was not depressed. The infrared spectra of the two preparations were identical.

C. *Via* Ethyl N-Carbobenzoxy-S-diphenylmethyl-L-cysteinyl-S-benzoyl-L-cysteinylglycinate (Id).—To a solution of 0.565 g (0.792 mmole) of Id in 16 ml of absolute ethanol was added 1.6 ml of 0.5 *N* sodium ethoxide in ethanol solution. The reaction mixture was shaken 15 min at room temperature and acidified with 1 ml of 2 *N* hydrochloric acid solution. The solvent was evaporated and the resulting syrup was dissolved in ethyl acetate. The solution was washed three times with water, dried, and evaporated. A single recrystallization of the residue from methanol afforded 0.39 g (80.9%) of XIV as a powder, mp 156–162°. Chromatography and recrystallization from a methanol–petroleum ether (bp 30–60°) mixture (recovery 71.6%, yield of pure product 58%) raised the melting point to 165–166.5°. A mixture melting point with a sample of XIV prepared as described in A was not depressed. The product had $[\alpha]^{23}D$ −19.5° (*c* 1.07, DMF).

D. *Via* Hydrochloric Acid.—A solution of 0.426 g (0.5 mmole) of Ia in 12 ml of glacial acetic acid was treated with 4 ml of 1 *N* hydrochloric acid solution. The solution was heated on the steam bath for 1.5 hr. The thin layer chromatogram (chloroform–ethyl acetate, 70:30) exhibited a spot due to triphenylmethyl chloride, XIV, and a faint (iodine) unidentified spot. The reaction mixture gave no color with ninhydrin.

When Ia was hydrolyzed under the same conditions, with 13% dry hydrogen chloride in acetic acid the thin layer chromatogram exhibited seven spots of similar intensity (iodine).

Ethyl N-Carbobenzoxy-S-triphenylmethyl-L-cysteinyl-S-diphenylmethyl-L-cysteinylglycinate (Ib).—To a solution of 6 g of hydrogen bromide in 30 ml of glacial acetic acid was added, portionwise, 15.2 g (0.03 mole) of XII.[5a] The solution was allowed to stand 30 min at room temperature, diluted with 500 ml of dry ether and 30 ml of petroleum ether, and chilled. The supernatant was decanted; the residue was washed with three 25-ml portions of dry ether and dried over calcium chloride and potassium hydroxide. The solid hydrobromide (XIII), 12.9 g, was dissolved in 150 ml of chloroform and treated with 16.2 g (0.28 mole) of N-carbobenzoxy-S-triphenylmethyl-L-cysteine N,N-diethylammonium salt[3] followed by 6.15 g (0.029 mole) of DCC. The mixture was stirred 12 hr at room temperature. The reaction mixture was filtered and the filtrate was evaporated to an oily residue which was dissolved in ethyl acetate. The solution was washed successively with 1 *N* hydrochloric acid solution, 5% sodium bicarbonate solution, and water. The dried organic layer was evaporated to a crystalline solid, Ib, which was recrystallized from absolute ethanol to yield 14.8 g (61.4%) of Ib as needles, mp 182–184° and 184–185° after further recrystallization, $[\alpha]^{24}D$ −9.7° (*c* 1.0, DMF).

Anal. Calcd for $C_{50}H_{49}N_3O_6S_2$: C, 70.41; H, 5.79; N, 4.94; S, 7.52. Found: C, 70.27; H, 5.93; N, 5.09; S, 7.28.

Ethyl N-Carbobenzoxy-L-cysteinyl-S-diphenylmethyl-L-cysteinylglycinate (XV). A. *Via* Mercury(II) Acetate.—To a warm solution of 4.26 g (5 mmoles) of Ib in a mixture of 100 ml of absolute ethanol and 30 ml of ethyl acetate was added a solution of 2.1 g (6.6 mmoles) of mercury(II) acetate in 30 ml of absolute ethanol. The mixture was kept at 43–47° for 1 hr. The usual work-up provided a crystalline solid which was recrystallized from a small volume of methanol to afford 0.92 g of XV as needles, mp 152–153°. A second crop, 0.24 g, mp 150–152°, was obtained from the mother liquor (total yield, 1.16 g, 38%): $[\alpha]^{23}D$ −26.1° (*c* 1.01, DMF).

Anal. Calcd for $C_{31}H_{35}N_3O_6S_2$: C, 61.15; H, 5.78; N, 6.89; S, 10.50. Found: C, 60.97; H, 5.93; N, 6.80; S, 10.47.

B. *Via* Silver Nitrate.—To a solution of 0.852 g (1.0 mmole) of Ib in 25 ml of ethyl acetate and 15 ml of absolute ethanol was added a solution of 0.17 g (1.0 mmole) of silver nitrate in 10 ml of absolute ethanol containing 0.08 ml of pyridine. The mixture was heated on the steam bath for 1.5 hr. Work-up in the usual manner provided the crude tripeptide, XV, which was purified by silicic acid chromatography and recrystallization from methanol–petroleum ether. The purified product was obtained as 0.10 g (16.4%) of needles, mp 151–153°, $[\alpha]^{23}D$ −25.5° (*c* 1.03, DMF). A mixture melting point with the sample obtained as described in A was not depressed.

Ethyl N-Carbobenzoxy-S-benzoyl-L-cysteinyl-S-triphenylmethyl-L-cysteinylglycinate (Ic).—To a solution of 2.44 g (5.03 mmoles) of crude VIII in 60 ml of chloroform was added 0.70 ml of triethylamine, followed by 1.86 g (5.04 mmoles) of IX[5b] and 1.15 g (5.53 moles) of DCC. The mixture was kept at room temperature for 12 hr, acidified with a few drops of acetic acid, and filtered. The filtrate was evaporated and the residue was dissolved in ethyl acetate. The organic layer was washed with successive portions of 1 *N* hydrochloric acid solution, 5% sodium bicarbonate solution, and water. The dried organic layer was evaporated and the resulting oil was crystallized by trituration with ether. Recrystallization of the solid from methanol provided 2.17 g (65.8%) of Ic as needles, mp 134–137°. The analytical sample had mp 137–139°, $[\alpha]^{24.5}D$ −26.8° (*c* 1.0, DMF).

Anal. Calcd for $C_{44}H_{43}N_3O_7S_2$: C, 66.90; H, 5.49; N, 5.32; S, 8.12. Found: C, 67.04; H, 5.47; N, 5.64; S, 8.27.

Ethyl N-Carbobenzoxy-L-cysteinyl-S-triphenylmethyl-L-cysteinylglycinate (XVI).—To a suspension of 2.03 g (2.58 mmoles) of Ic in 80 ml of absolute ethanol was added 5.30 ml

(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]

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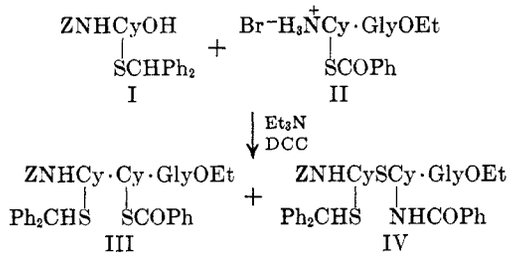
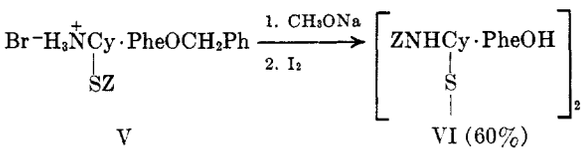
The S → N acyl migration of the S-benzoyl and S-carbobenzoxy groups has been studied. Migration of the benzoyl group occurs prior to coupling when N,N′-dicyclohexylcarbodiimide or *p*-nitrophenyl esters are employed. The direct coupling of methyl L-cysteinate with several N-protected amino acids has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

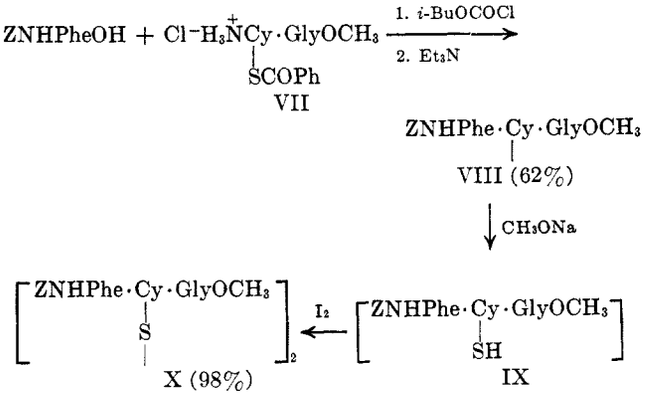


III were invariably obtained despite the fact that related derivatives were produced in reasonable yield. Since the substitution of II for other S-protected cysteine derivatives represented the major change in the reaction, the difficulty was believed to involve S → N benzoyl migration in the free base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