The 4-(Methylthio)phenyl and 4-(Methylsulfonyl)phenyl Esters in the Preparation of Peptides and Polypeptides.[1] Synthesis of the Protected Heptapeptide (A_{82}–A_{88}) of Bovine Chymotrypsinogen A

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The syntheses of some N-*t*-butyloxycarbonyl-L-amino acid pentachlorophenyl esters, prepared for facile peptide synthesis, are described. Their use is illustrated by the synthesis of the protected tripeptide N,N′-dicarbobenzoxy-L-lysyl-L-leucyl-ε-N-carbobenzoxy-L-lysine 4-(methylthio)phenyl ester which corresponds to the sequence (A_{82}–A_{84}) of bovine chymotrypsinogen A. Oxidation of this tripeptide derivative yielded the protected tripeptide 4-(methylsulfonyl)phenyl activated ester without rupture of the protecting groups or peptide bonds. By a similar method the protected tetrapeptide L-isoleucyl-L-alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine 4-(methylthio)-phenyl ester hydrochloride, sequence A_{85}–A_{88} of the same material, was prepared. The protected heptapeptide sequence (A_{82}–A_{88}) of bovine chymotrypsinogen A, N,N′-dicarbobenzoxy-L-lysyl-L-leucyl-ε-N-carbobenzoxy-L-lysyl-L-isoleucyl-L-alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine 4-(methylthio)phenyl ester, was obtained by coupling the protected tripeptide (A_{82}–A_{84}) with the tetrapeptide derivative (A_{85}–A_{88}) through the 4-(methylsulfonyl)-phenyl activated ester.

The use of a protective ester which can be converted into an ester activated toward aminolysis should be of great utility in peptide and polypeptide synthesis. For this purpose we have suggested[1] the use of the 4-(methylthio)phenyl ester (MTP) as a carboxyl protecting group which can be easily converted into the 4-(methylsulfonyl)phenyl activated ester (MSO_2P). The MTP esters have the following important properties: they can be easily prepared by the N,N′-dicyclohexylcarbodiimide (DCC) method;[3] the N-carbobenzoxy and N-t-butyloxycarbonyl protecting groups can be easily removed in their presence; and mild oxidation, even in the presence of the N-carbobenzoxy and *t*-butyl ester protecting groups, yields the activated MSO_2P esters.

To extend the utility of this method for peptide synthesis, it was necessary to see if the conversion of the protective MTP ester into the MSO_2P activated ester could be achieved on a larger peptide, and also to utilize this activated ester to couple blocks of peptides together. To this end the synthesis of the protected heptapeptide N,N′-dicarbobenzoxy-L-lysyl-L-leucyl-ε-N-carbobenzoxy-L-lysyl-L-isoleucyl-L-alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine 4-(methylthio)phenyl ester (**1**),

Z-Lys-Leu-Lys-Ile-Ala-Lys-Val-OMTP
Z Z Z

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corresponding to the sequence A_{82}–A_{88} of bovine chymotrypsinogen A,[4] is described.

The approach used was to prepare the protected tripeptide (A_{82}–A_{84}), N,N′-dicarbobenzoxy-L-lysyl-L-leucyl-ε-N-carbobenzoxy-L-lysine 4-(methylsulfonyl)-phenyl ester (**2**) and couple it to the tetrapeptide derivative (A_{85}–A_{88}), L-isoleucyl-L-alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine 4-(methylthio)phenyl ester hydrochloride (**3**). For the facile synthesis of both, the protected tripeptide and tetrapeptide blocks of N-*t*-butyloxycarbonyl-L-amino acid pentachlorophenyl esters were used.[5] They are easily prepared from the N-*t*-butyloxycarbonyl-L-amino acid and pentachlorophenol by the use of N,N′-dicyclohexylcarbodiimide.[3] The resulting esters are far more stable than the corresponding N-hydroxysuccinimide esters and are of higher melting point than the *p*-nitrophenyl esters, both of which have been used for rapid peptide synthesis.[6,7] The physical constants of a number of N-*t*-butyloxycarbonyl-L-amino acid pentachlorophenyl esters are given in the Experimental Section.

N-*t*-Butyloxycarbonyl-ε-N-carbobenzoxy-L-lysine 4-(methylthio)phenyl ester (**4**) was prepared in good yield from N-*t*-butyloxycarbonyl-ε-N-carbobenzoxy-L-lysine and 4-(methylthio)phenol by condensation with N,N′-dicyclohexylcarbodiimide. Treatment of **4** with 1 *N* hydrogen chloride in glacial acetic acid afforded ε-N-carbobenzoxy-L-lysine 4-(methylthio)phenyl ester hydrochloride (**5**). N-*t*-Butyloxycarbonyl-L-leucine pentachlorophenyl ester was coupled to **5** in methylene chloride to yield the protected dipeptide N-*t*-butyloxycarbonyl-L-leucyl-ε-N-carbobenzoxy-L-lysine 4-(methylthio)phenyl ester (**6**). The N-*t*-butyloxycarbonyl protecting group was removed from **6** by treatment with 1 *N* HCl–acetic acid to give L-leucyl-ε-N-carbobenzoxy-L-lysine 4-(methylthio)phenyl ester hydrochloride (**7**). N,N′-Dicarbobenzoxy-L-lysine pentachlorophenyl ester[5] was coupled to **7** to yield the blocked tripeptide N,N′-dicarbobenzoxy-L-lysyl-L-leucyl-ε-N-carbobenzoxy-L-lysine 4-(methylthio)phenyl ester (**8**). Oxidation of **8** using 30% hydrogen peroxide in glacial acetic acid for a period of 12 hr gave, without appreciable decomposition, the protected tripeptide activated ester N,N′-dicarbobenzoxy-L-leucyl-ε-N-carbobenzoxy-L-lysine 4-(methylsulfonyl)phenyl ester (**2**) in good yield. For the synthesis of the protected tetrapeptide, 4-(methylthio)-phenyl ester hydrochloride **3**, N-*t*-butyloxycarbonyl-L-valine MTP ester (**9**) was prepared by the DCC condensation of N-*t*-butyloxycarbonyl-L-valine and 4-(methylthio)phenol. The blocking N-*t*-butyloxycarbonyl group was removed by treatment with 1 *N*

(1) This is the second article in this series. See B. J. Johnson and P. M. Jacobs, *Chem. Commun.*, 73 (1968), for previous paper.

(2) To whom any correspondence should be sent.

(3) J. C. Sheehan and G. P. Hess, *J. Amer. Chem. Soc.*, **77**, 1067 (1955).

(4) B. S. Hartley, *Nature*, **201**, 1285 (1964).

(5) J. Kovacs, M. Q. Ceprini, C. A. Dupraz, and G. N. Schmit, *J. Org. Chem.*, **32**, 3696 (1967), and other references cited therein. These authors have suggested and used N-carbobenzoxy-L-amino acid pentachlor ophenyl esters for the synthesis of peptides.

(6) D. A. Laufer and E. R. Blout, *J. Amer. Chem. Soc.*, **89**, 1246 (1967).

(7) M. Bodanszky, M. A. Ondetti, S. D. Levine, and N. J. William s, *ibid.*, **89**, 6753 (1967).

hydrogen chloride in glacial acetic acid to give L-valine MTP ester hydrochloride (**10**) in good yield. The hydrochloride **10** was coupled to N-*t*-butyloxycarbonyl-ε-N-carbobenzoxy-L-lysine pentachlorophenyl ester to give the blocked dipeptide N-*t*-butyloxycarbonyl-ε-N-carbobenzoxy-L-lysyl-L-valine MTP ester (**11**). Treatment of **11** with 1 *N* HCl–acetic acid gave ε-N-carbobenzoxy-L-lysyl-L-valine MTP ester hydrochloride (**12**). The protected dipeptide was lengthened by treating it first with N-*t*-butyloxycarbonyl-L-alanine pentachlorophenyl ester to give N-*t*-butyloxycarbonyl-L-alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine MTP ester (**13**) and then 1 *N* HCl–glacial acetic acid to produce L-alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine MTP ester hydrochloride (**14**). Further reaction of **14** with N-*t*-butyloxycarbonyl-L-isoleucine pentachlorophenyl ester produced the blocked tetrapeptide N-*t*-butyloxycarbonyl-L-isoleucyl-L-alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine MTP ester (**15**). Finally, reaction of **15** with 1 *N* HCl–glacial acetic acid gave the protected tetrapeptide hydrochloride, L-isoleucyl-L-alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine 4-(methylthio)phenyl ester hydrochloride (**3**), in good yield.

To prepare the protected heptapeptide corresponding to the sequence A_{82}–A_{88} of bovine chymotrypsinogen A, N,N′-dicarbobenzoxy-L-lysyl-L-leucyl-ε-N-carbobenzoxy-L-lysyl-L-isoleucyl-L-alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine MTP ester (**1**), it was necessary to couple the blocked tetrapeptide hydrochloride **3** to the protected tripeptide N,N′-dicarbobenzoxy-L-lysyl-L-leucyl-ε-N-carbobenzoxy-L-lysine MSO_2P ester (**2**). With other methods of preparing peptides, the presence of salts of tertiary amines has been shown to increase the degree of racemization of the amino acid residue carrying the activated moiety.[8] Thus to minimize this possibility of racemization occurring the free amine of **3** was added to the protected tripeptide **2** to give the fully protected heptapeptide **1**. The optical purity of the protected heptapeptide was calculated to be 97.1 ± 5% by determining the optical activity of the acid hydrolysate and comparing it with that of a control.

It would appear from this work that the N-*t*-butyloxycarbonyl derivatives of amino acid pentachlorophenyl esters are useful intermediates for the facile synthesis of peptide blocks. It has also been shown that the protective 4-(methylthio)phenyl ester can be converted into the activated 4-(methylsulfonyl)phenyl ester without decomposition of the fully protected tripeptide to which it was attached. Also, the 4-(methylsulfonyl)phenyl ester is of use for the coupling of blocks of peptides together without appreciable racemization occurring under the coupling conditions used. It is anticipated that this method of activation will be of little use for peptides which include the amino acid residues, methionine, cysteine, cystine, and possibly tryptophan.

Experimental Section[9]

General Procedure for the Preparation of N-*t*-Butyloxycarbonyl-L-amino Acid Pentachlorophenyl Esters.—The general procedure of preparation of these esters is illustrated by the preparation of **N-*t*-butyloxycarbonyl-ε-N-carbobenzoxy-L-lysine pentachlorophenyl ester.** To a solution of N-*t*-butyloxycarbonyl-ε-N-carbobenzoxy-L-lysine[10] (11.5 g, 30.3 mmol) and pentachlorophenol (8.06 g, 30.3 mmol) in methylene chloride (100 ml) was added dicyclohexylcarbodiimide (6.86 g, 33.3 mmol). The mixture was stirred at room temperature overnight. The solid N,N′-dicyclohexylurea was filtered off, and the filtrate was concentrated under reduced pressure. The solid product was dissolved in ethyl acetate (300 ml), and the insoluble urea was filtered off. The filtrate was washed with 10% citric acid solution (100 ml), water (100 ml), 1 *N* sodium hydrogen carbonate solution (100 ml), and water (two 100-ml portions). The solution was dried (Na_2SO_4), and the solvent was removed under reduced pressure to give the solid pentachlorophenyl ester. It was crystallized from methanol (yield 14.0 g, 73.5%), mp 140–141°. Further recrystallization from ethyl acetate–hexane increased the sharpness of the melting point to 141°, $[\alpha]^{29}D$ −15.0° (*c* 4.99, chloroform).

Anal. Calcd for $C_{25}H_{27}Cl_5N_2O_6$: C, 47.8; H, 4.3; N, 4.5. Found: C, 47.7; H, 4.5; N, 4.7.

The following compounds were also prepared by the same method: *t*-butyloxycarbonyl-L-alanine pentachlorophenyl ester [mp 166°, $[\alpha]^{25}D$ −22.2° (*c* 5.12, chloroform) (*Anal.* Calcd for $C_{14}H_{14}Cl_5NO_4$: C, 38.45; H, 3.2; Cl, 4.05. Found: C, 38.7; H, 3.4; Cl, 40.3)]; *t*-butyloxycarbonylglycine pentachlorophenyl ester [mp 142° (*Anal.* Calcd for $C_{13}H_{12}Cl_5NO_4$: C, 36.8; H, 3.0; Cl, 41.8. Found: C, 37.0; H, 2.9; Cl, 42.0)]; *t*-butyloxycarbonyl-L-isoleucine pentachlorophenyl ester [mp 119°, $[\alpha]^{29}D$ −28.0° (*c* 5.18, ethyl acetate) (*Anal.* Calcd for $C_{17}H_{20}Cl_5NO_4$: C, 42.6; H, 4.2; Cl, 36.95. Found: C, 42.6; H, 4.25; Cl, 37.05)]; *t*-butyloxycarbonyl-L-leucine pentachlorophenyl ester[11] [mp 111°, $[\alpha]^{29}D$ −35.3° (*c* 4.95, ethyl acetate) (*Anal.* Calcd for $C_{17}H_{20}Cl_5NO_4$: C, 42.6; H, 4.2; Cl, 36.95. Found: C, 42.6; H, 4.25; Cl, 37.05)]; *t*-butyloxycarbonyl-L-phenylalanine pentachlorophenyl ester[11] [mp 148°, $[\alpha]^{29}D$ −47.1° (*c* 4.82, ethyl acetate) (*Anal.* Calcd for $C_{20}H_{18}Cl_5NO_4$: C, 46.8; H, 3.5; Cl, 34.5. Found: C, 46.8; H, 3.4; Cl, 34.5)]; *t*-butyloxycarbonyl-O-benzyl-L-tyrosine pentachlorophenyl ester [mp 142°, $[\alpha]^{29}D$ −26.8° (*c* 4.98, chloroform) (*Anal.* Calcd for $C_{27}H_{24}Cl_5NO_5$: C, 52.3; H, 3.9; Cl, 28.6. Found: C, 52.25; H, 3.5; Cl, 28.65)]; *t*-butyloxycarbonyl-L-valine[11] pentachlorophenyl ester [mp 126°, $[\alpha]^{29}D$ −38.1° (*c* 5.00, ethyl acetate) (*Anal.* Calcd for $C_{16}H_{18}Cl_5NO_4$: C, 41.3; H, 3.9; Cl, 38.1. Found: C, 41.5; H, 4.0; Cl, 38.4)]; N,N′-di-*t*-butyloxycarbonyl-L-lysine pentachlorophenyl ester [mp 148°, $[\alpha]^{25}D$ −20.6° (*c* 4.6, dimethylformamide) (*Anal.* Calcd for $C_{22}H_{29}Cl_5N_2O_6$: C, 44.4; H, 4.9; Cl, 29.8. Found: C, 44.7; H, 4.9; Cl, 29.8)].

N-*t*-Butyloxycarbonyl-ε-N-carbobenzoxy-L-lysine 4-(Methylthio)phenyl Ester (4).—To a solution of N-*t*-butyloxycarbonyl-ε-N-carbobenzoxy-L-lysine (9.0 g, 0.0237 mol) in methylene chloride (150 ml) was added 6.12 g of DCC; after stirring for 5 min at room temperature 3.32 g of 4-(methylthio)phenol was added. The reaction mixture was stirred overnight. The precipitated urea was filtered off, and the solvent was evaporated to give an oil. The oil was dissolved in ethyl acetate and washed with sodium bicarbonate solution (100 ml) and water (two 150-ml portions), dried (Na_2SO_4), and evaporated under reduced pressure to give a solid. This was recrystallized from ethyl acetate–hexane to yield the 4-(methylthio)phenyl ester (10.1 g, 85%): mp 96–97°; $[\alpha]^{25}D$ −21.6° (*c* 2.4, dimethylformamide).

Anal. Calcd for $C_{26}H_{34}N_2O_6S$: C, 62.1; H, 6.8; N, 5.6. Found: C, 62.2; H, 6.9; N, 5.5.

ε-N-Carbobenzoxy-L-lysine 4-(Methylthio)phenyl Ester Hydrochloride (5).—N-*t*-Butyloxycarbonyl-ε-N-carbobenzoxy-L-lysine 4-(methylthio)phenyl ester (9.5 g, 0.0189 mol) was added to 57 ml of 1 *N* hydrogen chloride in glacial acetic acid and left at room temperature for 30 min. The hydrochloride was precipitated by the addition of anhydrous ether and filtered off. It was recrystallized from methanol–ether to yield 7.9 g (95%): mp 147°; $[\alpha]^{25}D$ +18.0° (*c* 4.8, methanol).

Anal. Calcd for $C_{21}H_{27}ClN_2O_4S$: C, 57.4; H, 6.2; N, 6.4. Found: C, 57.1; H, 6.3; N, 6.1.

N-*t*-Butyloxycarbonyl-L-leucyl-ε-N-carbobenzoxy-L-lysine 4-(Methylthio)phenyl Ester (6).—ε-N-Carbobenzoxy-L-lysine 4-(methylthio)-phenyl ester hydrochloride (3.65 g, 0.008 mol) was added to a solution of N-*t*-butyloxycarbonyl-L-leucine pentachlo-

(8) M. W. Williams and G. T. Young, *J. Chem. Soc.*, 3701 (1964).

(9) All melting points are uncorrected. Analyses were carried out by either Dr. S. N. Nagy of Massachusetts Institute of Technology, Cambridge, Mass., or the Galbraith Laboratories, Inc., Knoxville, Tenn. Optical rotations were taken on a Carl Zeiss precision polarimeter. Thin layer chromatography employed Silicar TLC-7G as support, methanol–chloroform (1:9) as solvent, and iodine for detection purposes.

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

(11) A. J. Corcoran, this laboratory.

rophenyl ester (4.0 g, 0.008 mol) in methylene chloride (50 ml) containing 0.85 g of triethylamine. The solution was stirred at room temperature overnight and then evaporated under reduced pressure to give a solid. This solid was suspended in ethyl acetate and washed with 10% citric acid solution (50 ml) and water (three 200-ml portions), dried (Na_2SO_4), and evaporated to yield a solid. Recrystallization of this crude material from ethyl acetate–hexane yielded the dipeptide 4-(methylthio)-phenyl ester (3.9 g, 77%): mp 87°; $[\alpha]^{24}D$ −21.3° (*c* 6.7, dimethylformamide).

Anal. Calcd for $C_{32}H_{45}N_3O_7S$: C, 62.4; H, 7.4; N, 6.8. Found: C, 62.3; H, 7.55; N, 6.6.

L-Leucyl-ε-N-Carbobenzoxy-L-lysine 4-(Methylthio)phenyl Ester Hydrochloride (7).—N-*t*-Butyloxycarbonyl-L-leucyl-ε-N-carbobenzoxy-L-lysine 4-(methylthio)phenyl ester (3.5 g, 0.00568 mol) was added to 17 ml of 1 *N* hydrogen chloride in glacial acetic acid and left at room temperature for 35 min. Addition of anhydrous ether to the reaction mixture precipitated the hydrochloride (2.9 g, 92%): mp 185°; $[\alpha]^{24}D$ −7.7° (*c* 5, methanol).

Anal. Calcd for $C_{27}H_{38}ClN_3O_6S$: C, 58.7; H, 6.9; N, 7.6. Found: C, 58.9; H, 7.1; N, 7.6.

N,N′-Dicarbobenzoxy-L-lysyl-L-leucyl-ε-N-carbobenzoxy-L-lysine 4-(Methylthio)phenyl Ester (8).—To a solution of N,N′-dicarbobenzoxy-L-lysine pentachlorophenyl ester[5] (3.03 g 0.00457 mol) in dimethylformamide (20 ml) containing 0.5 g of triethylamine was added 2.5 g (0.00453 mol) of L-leucyl-ε-N-carbobenzoxy-L-lysine 4-(methylthio)phenyl ester hydrochloride. The mixture was stirred overnight at room temperature and then poured into 300 ml of water. The precipitated material was filtered off, dried, and crystallized from ethyl acetate–ether to yield the protected tripeptide (2.9 g, 70%): mp 153°; $[\alpha]^{25}D$ −34.5° (*c* 5.8, dimethylformamide).

Anal. Calcd for $C_{49}H_{61}N_5O_{10}S$: C, 64.5; H, 6.7; N, 7.7. Found: C, 64.5; H, 6.8; N, 7.5.

N,N′-Dicarbobenzoxy-L-lysyl-L-leucyl-ε-N-carbobenzoxy-L-lysine 4-(Methylsulfonyl)phenyl Ester (2).—To N,N′-Dicarbobenzoxy-L-lysyl-L-leucyl-ε-N-carbobenzoxy-L-lysine 4-(methylthiophenyl ester (2.5 g, 0.00274 mol) dissolved in 100 ml of glacial acetic acid was added 10 ml of 30% hydrogen peroxide solution. The mixture was left at room temperature for 12 hr and then evaporated under reduced pressure to a small volume. Addition of water afforded a white precipitate, which was filtered off, dried, and chromatographed on a column of silicic acid (pH 7) using chloroform as eluent. The major fraction was crystallized from ethyl acetate–ether to give the protected tripeptide active ester (2.0 g, 77%): mp 179°; $[\alpha]^{24}D$ −33.0° (*c* 3.8, dimethylformamide); R_f 0.69.

Anal. Calcd for $C_{49}H_{61}N_5O_{12}S$: C, 62.3; H, 6.5; N, 7.4. Found: C, 62.2; H, 6.8; N, 7.4.

N-*t*-Butyloxycarbonyl-L-valine 4-(Methylthio)phenyl Ester (9).—To a solution of N-*t*-butyloxycarbonyl-L-valine (8.7 g, 0.04 mol) in 100 ml of methylene chloride was added 8.3 g of DCC; solution was stirred at room temperature for 10 min. 4-(Methylthio)phenol (5.6 g) was added to the solution, and stirring was continued overnight. The precipitated urea was filtered off, and the filtrate evaporated to give an oil. The oil was dissolved in ethyl acetate, washed with sodium bicarbonate solution and water, and then dried (Na_2SO_4). Removal of the solvent under reduced pressure gave an oil. This oil was chromatographed on a column of Silicar CC-7 (pH 7) using chloroform as the eluent. The major fraction was collected and evaporated to give the 4-(methylthio)phenyl ester as an oil (8.5 g, 62.5%): $[\alpha]^{25}D$ −6.1° (*c* 8.2, in dimethylformamide); ν_{max}^{Nujol} 1755 cm^{-1} (C=O ester).

Anal. Calcd for $C_{17}H_{25}NO_4S$: C, 60.2; H, 7.4. Found: C, 60.35; H, 7.7.

L-Valine 4-(Methylthio)phenyl Ester Hydrochloride (10).—To N-*t*-butyloxycarbonyl-L-valine 4-(methylthio)phenyl ester (8.6 g, 0.0254 mol) was added 76 ml of 1 *N* hydrogen chloride in glacial acetic acid. The solution was left at room temperature for 30 min and then poured into anhydrous ether. The precipitated hydrochloride was collected and crystallized from methanol–ether to give 5.5 g (79%): mp 220° dec; $[\alpha]^{28}D$ +18.9° (*c* 1.3, methanol).

Anal. Calcd for $C_{12}H_{18}ClNO_2S$: C, 52.3; H, 6.6; S, 11.6. Found: C, 52.3; H, 6.75; S, 11.8.

N-*t*-Butyloxycarbonyl-ε-N-carbobenzoxy-L-lysyl-L-valine 4-(Methylthio)phenyl Ester (11).—To a solution of 6.29 g (0.01 mol) of N-*t*-butyloxycarbonyl-ε-N-carbobenzoxy-L-lysine pentachlorophenyl ester and 2.76 g (0.01 mol) of L-valine 4-(methylthio)phenyl ester hydrochloride in 50 ml of methylene chloride was added 1 g of triethylamine. The solution was stirred overnight at room temperature. The solvent was evaporated, and the residue was suspended in ethyl acetate and washed with 10% citric acid and then water. The organic extracts were dried (Na_2SO_4) and then evaporated to give a solid. This material was crystallized from ethyl acetate–hexane to give 5.1 g (85%) of the protected dipeptide: mp 93°; $[\alpha]^{25}D$ −5.3° (*c* 1.1, methanol); R_f 0.78.

Anal. Calcd for $C_{31}H_{43}N_3O_7S$: C, 61.85; H, 7.2; N, 7.0. Found: C, 62.1; H, 7.3; N, 6.9.

ε-N-Carbobenzoxy-L-lysyl-L-valine 4-(Methylthio)phenyl Ester Hydrochloride (12).—To 3.0 g (0.005 mol) of N-*t*-butyloxycarbonyl-ε-N-carbobenzoxy-L-lysyl-L-valine 4-(methylthio)-phenyl ester was added 14 ml of 1 *N* hydrogen chloride in glacial acetic acid, and the solution was left at room temperature for 30 min. Addition of dry ether precipitated the hydrochloride which was recrystallized from methanol–ether to yield 2.5 g (93%) of pure product: mp 145°; $[\alpha]^{26}D$ −8.6° (*c* 4.1, methanol).

Anal. Calcd for $C_{26}H_{36}ClN_3O_5S$: C, 58.0; H, 6.7; N, 7.8. Found: C, 57.8; H, 7.0; N, 7.75.

N-*t*-Butyloxycarbonyl-L-alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine 4-(Methylthio)phenyl Ester (13).—To N-*t*-butyloxycarbonyl-L-alanine pentachlorophenyl ester (1.63 g, 0.00305 mol) dissolved in 50 ml of methylene chloride was added 2.0 g (0.00373 mol) of ε-N-carbobenzoxy-L-lysyl-L-valine 4-(methylthio)phenyl ester hydrochloride and 0.4 g of triethylamine. The solution was stirred overnight at room temperature. The solvent was removed *in vacuo*, and the residue was suspended in ethyl acetate. This was washed with 10% citric acid solution and then water, dried (Na_2SO_4), and evaporated under reduced pressure to give a solid. This material was run through a short column of Silicar CC-7 using chloroform as eluent to yield 2.1 g (84%) of the protected tripeptide: mp 151°; $[\alpha]^{26}D$ −35.0° (*c* 7, dimethylformamide); R_f 0.71.

Anal. Calcd for $C_{34}H_{48}N_4O_8S$: C, 60.7; H, 7.2; N, 8.3. Found: C, 60.8; H, 7.5; N, 8.2.

L-Alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine 4-(Methylthio)-phenyl Ester Hydrochloride (14).—N-*t*-Butyloxycarbonyl-L-alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine 4-(methylthio)phenyl ester (2.0 g, 0.00297 mol) was added to 9 ml of 1 *N* hydrogen chloride in glacial acetic acid. The solution was left at room temperature for 35 min. Addition of dry ether precipitated 1.5 g (83%) of the hydrochloride: mp 193°; $[\alpha]^{27}D$ −33.1° (*c* 4.9, methanol).

Anal. Calcd for $C_{29}H_{41}ClN_4O_6S$: C, 57.2; H, 6.8; N, 9.2. Found: C, 57.2; H, 6.9; N, 9.0.

N-*t*-Butyloxycarbonyl-L-isoleucyl-L-alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine 4-(Methylthio)phenyl Ester (15).—N-*t*-Butyloxycarbonyl-L-isoleucine pentachlorophenyl ester (0.8 g, 0.00166 mol) was dissolved in 50 ml of methylene chloride and 1.0 g (0.00165 mol) of L-alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine 4-(methylthio)phenyl ester hydrochloride was added. To this mixture 0.17 g of triethylamine was introduced, and the solution was stirred overnight. The solvent was removed, and the residue was dissolved in ethyl acetate and washed with 10% citric acid and then water, dried (Na_2SO_4), and evaporated to give a solid. This material was crystallized from ethyl acetate–hexane to give 0.9 g (76%) of the protected tetrapeptide: mp 188–189°; $[\alpha]^{24}D$ −28.9° (*c* 2.6, dimethylformamide); R_f 0.65.

Anal. Calcd for $C_{40}H_{59}N_5O_9S$: C, 61.1; H, 7.6; N, 8.9. Found: C, 61.2; H, 7.7; N, 9.1.

L-Isoleucyl-L-alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine 4-Methylthio)phenyl Ester Hydrochloride (3).—To 2 ml of 1 *N* hydrogen chloride in glacial acetic acid was added 0.5 g (0.00064 mol) of N-*t*-butyloxycarbonyl-L-isoleucyl-L-alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine 4-(methylthio)phenyl ester, and the resulting mixture was left at room temperature for 50 min. Addition of dry ether precipitated 0.41 g (89%) of the hydrochloride: mp 230° dec; $[\alpha]^{25}D$ −38.6° (*c* 5.8, methanol).

Anal. Calcd for $C_{35}H_{52}ClN_5O_7S$: C, 58.2; H, 7.3; N, 9.7. Found: C, 58.1; H, 7.4; N, 9.4.

N,N′-Dicarbobenzoxy-L-lysyl-L-leucyl-ε-N-carbobenzoxy-L-lysyl-L-isoleucyl-L-alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine 4-(Methylthio)phenyl Ester (1).—L-Isoleucyl-L-alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine 4-(methylthio)phenyl ester hydrochloride (0.4 g, 0.000554 mol) was suspended in 10 ml of ethyl acetate and 0.05 g of triethylamine was added. The precipitated triethylamine hydrochloride was removed by filtration. The filtrate was added to a solution of 0.55 g of N,N′-dicarbobenzoxy-L-

lysyl-L-leucyl-ε-N-carbobenzoxy-L-lysine 4-(methylsulfonyl)-phenyl ester in 10 ml of dimethylformamide, and the resulting solution was stirred overnight. This was poured into 300 ml of water containing 50 ml of 10% citric acid solution and stirred for 2 hr. The precipitate was filtered off, dried, and crystallized from chloroform–ether to yield 0.6 g (73%) of the fully protected heptapeptide: mp 118–120°; $[\alpha]^{25}D$ −25° (*c* 1.0, dimethylformamide); R_f 0.49.

Anal. Calcd for $C_{77}H_{104}N_{10}O_{16}S$: C, 62.1; H, 7.0; N, 9.4. Found: C, 62.4; H, 7.1; N, 9.3.

Optical Purity of N,N′-Dicarbobenzoxy-L-lysyl-L-leucyl-ε-N-carbobenzoxy-L-lysyl-L-isoleucyl-L-alanyl-ε-N-carbobenzoxy-L-lysyl-L-valine 4-(Methylthio)phenyl Ester (1).—The protected heptapeptide **1** (0.078 g, 5.237×10^{-5} mol) was dissolved in 10 ml of 6 *N* hydrochloric acid–glacial acetic acid (1:1) and heated under reflux at 100–105° for 24 hr. The solution was evaporated to dryness, and the residue was dissolved in 6 *N* hydrochloric acid–glacial acetic acid (4:1) so that the final volume was 2 ml: $[\alpha]^{30}D$ +28.69° (calculated on the basis of the expected amounts of lysine, leucine, isoleucine, alanine, and valine).

A control of 0.0073 g of 4-(methylthio)phenol, 0.0287 g of lysine hydrochloride, 0.0069 g of L-leucine, 0.0069 g of L-isoleucine, 0.0046 g of L-alanine, 0.0061 g of L-valine, 0.0056 g of benzyl alcohol, and 10 ml of 6 *N* hydrochloric acid–glacial acetic acid (1:1) was heated simultaneously with and under the same conditions as those used for the protected heptapeptide **1**. After 24 hr the solution was evaporated to dryness and made up to 2 ml with 6 *N* hydrochloric acid–glacial acetic acid (4:1), $[\alpha]^{30}D$ +29.54°, to give an optical purity of 97.1 ± 5%.

Registry No.—**1**, 17693-03-7; **2**, 17693-04-8; **3**, 17693-05-9; **4**, 17693-06-0; **5**, 17693-07-1; **6**, 17693-08-2; **7**, 17743-96-3; **8**, 17693-09-3; **9**, 17693-10-6; **10**, 17693-11-7; **11**, 17693-12-8; **12**, 17693-13-9; **13**, 17743-97-4; **14**, 17693-14-0; **15**, 17693-15-1; N-*t*-butyloxycarbonyl-ε-N-carbobenzoxy-L-lysine pentachlorophenyl ester, 17693-16-2; *t*-butyloxycarbonyl-L-alanine pentachlorophenyl ester, 17693-17-3; *t*-butyloxycarbonylglycine pentachlorophenyl ester, 17693-18-4; *t*-butyloxycarbonyl-L-isoleucine pentachlorophenyl ester, 17693-19-5; *t*-butyloxycarbonyl-L-leucine pentachlorophenyl ester, 17693-20-8; *t*-butyloxycarbonyl-L-phenylalanine pentachlorophenyl ester, 17693-21-9; *t*-butyloxycarbonyl-O-benzyl-L-tyrosine pentachlorophenyl ester, 17693-22-0; *t*-butyloxycarbonyl-L-valine pentachlorophenyl ester, 17693-23-1; N,N′-di-*t*-butyl oxycarbonyl-L-lysine pentachlorophenyl ester 17693-24-2.

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Notes

The 4-(Methylsulfonyl)phenyl Activated Ester. Susceptibility to Racemization[1]

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In a previous communication[3] it was shown that N-protected amino acid or peptide 4-(methylthio)phenyl esters could be converted by oxidation into 4-(methylsulfonyl)phenyl esters, which were sufficiently activated to be used in peptide synthesis. However, to evaluate the utility of this method, it was necessary to investigate the susceptibility of the activated ester to racemization. Since the most common mechanism is thought to be racemization through the oxazolone, Young's model[4] was chosen for study, because it is especially susceptible to racemization in this manner.

N-*t*-Butyloxycarbonyl-L-leucine 4-(methylthio)phenyl ester (**1**), was prepared from N-*t*-butyloxycarbonyl-L-leucine and 4-(methylthio)phenol using DCC. Treatment of **1** with hydrogen chloride in glacial acetic acid yielded L-leucine 4-(methylthio)phenyl ester hydrochloride (**2**), which was benzoylated to give N-benzoyl-L-leucine 4-(methylthio)phenyl ester (**3**). Oxidation of **3** with excess hydrogen peroxide in glacial acetic acid for 12 hr gave N-benzoyl-L-leucine 4-(methylsulfonyl)phenyl ester (**4**). Under these oxidative conditions the 4-(methylthio)phenyl ester is converted completely[3] into the 4-(methylsulfonyl)-phenyl ester as shown by infrared data. The presence of the optically active 4-(methylsulfinyl)phenyl ester was inferred to be absent. Thus it was considered that a comparison of the optical activity of the total acid hydrolysate of compounds **3** and **4** would indicate the amount of optical retention during this conversion. To this end N-benzoyl-L-leucine 4-(methylthio)phenyl ester (**3**) and N-benzoyl-L-leucine 4-(methylsulfonyl)-phenyl ester (**4**) were hydrolyzed using 6 *N* hydrochloric acid–glacial acetic acid (1:1) mixture, under identical conditions. Comparison of the the specific rotations of the hydrolysates of **3** and **4** showed that nearly 100% optical purity had been maintained.

In order to study the susceptibility of the 4-(methylsulfonyl)phenyl-activated ester to racemization in the presence of base, solutions of the ester **4** and tertiary amine (in 1:2 molar ratio) were mixed together in a 1-dm polarimeter tube; changes in optical rotation were observed on a Carl Zeiss polarimeter.

The general mechanism proposed[5,6] for racemization through the formation of an oxazolone provides the rate expression

$$\frac{-d[L]}{dt} = k_1[B]([L] - [D])$$

(1) This is the third article in this series. For the previous paper see B. J. Johnson and E. G. Trask, *J. Org. Chem.*, **33**, 4521 (1968).

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(3) B. J. Johnson and P. M. Jacobs, *Chem. Commun.*, 73 (1968).

(4) M. W. Williams and G. T. Young, *J. Chem. Soc.*, 881 (1963).

(5) M. Goodman and L. Levine, *J. Amer. Chem. Soc.*, **86**, 2918 (1964). M. Goodman and W. J. McGahren, *ibid.*, **87**, 3028 (1965).

(6) M. W. Williams and G. T. Young, *J. Chem. Soc.* 3701 (1964).