Pentachlorophenyl Esters of N-Carbobenzoxy-L-amino Acids

J. KOVACS, M. Q. CEPRINI, C. A. DUPRAZ, AND G. N. SCHMIT

Department of Chemistry, St. John's University, Jamaica, New York 11432

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Among the various active esters which have been used recently for peptide synthesis, the pentachlorophenyl (PCPOH) active ester, first reported in the literature[1] in 1961, was developed in this laboratory for peptide synthesis. Our previous papers[2-9] reported the preparation and polymerization of a few amino acid and peptide PCPOH esters.

PCPOH esters have the following important properties: they are among the most active esters;[10] peptide PCPOH esters can be prepared by the N,N'-dicyclohexylcarbodiimide (DCC) method with little or no racemization, as demonstrated by the Anderson and Young tests;[11-13] these esters can be easily used in trifunctional amino acids in combination with carbobenzoxy and *t*-butyl protecting groups;[9] and they are frequently higher melting compounds than other active esters, which makes them easier to purify by recrystallization.[14] Since these PCPOH esters proved to be useful in peptide synthesis, it was considered worthwhile to report the preparation of the PCPOH esters of most of the common N-carbobenzoxy-L-amino acids.

The most significant result of this work is that the PCPOH esters of N-carbobenzoxynitro-L-arginine and N-carbobenzoxy-L-serine could be prepared easily in a pure, stable form.[15] Previous attempts to synthesize the *p*-nitrophenyl (NPOH) and 2,4-dinitrophenyl (DNPOH) esters of N-carbobenzoxynitro-L-arginine resulted in either the formation of the lactam[16,17] or an impure product,[17-19] while N-carbobenzoxy-L-serine NPOH and DNPOH esters were reported only recently in unknown yield.[19]

N-Carbobenzoxyamino acid PCPOH esters were most conveniently prepared by the DCC method, although they could also be prepared through the mixed anhydride. Table I summarizes the reaction conditions and physical constants of these N-carbobenzoxyamino acid PCPOH esters.

N-Carbobenzoxynitro-L-arginine PCPOH ester was prepared by the usual procedure in good yield, in contrast with the NPOH ester. In a previous paper[13] it was shown that, during the preparation of N-carbobenzoxyglycine PCPOH ester from N-carbobenzoxyglycine, DCC, and PCPOH, the ester was formed preferentially through direct attack by PCPOH on the acylisourea intermediate I and to a smaller extent through N-carbobenzoxyglycine anhydride. When NPOH is used as the phenol derivative, however, the reaction proceeds to a greater extent through the anhydride intermediate. It is concluded that the weaker nucleophile, PCPOH, which is the stronger acid, reacts faster with the acylisourea derivative (Scheme I).

Applying this mechanism to nitroarginine, then the strongly acidic phenol, PCPOH, would protonate the acylisourea (I → II), producing an inductive effect which facilitates ester formation. In addition, protonation of the guanidino group by PCPOH would decrease the possibility of lactam formation.

N-Carbobenzoxy-L-serine PCPOH ester was also obtained in good yield, without protection of the side-chain hydroxyl. The unavailability of the corresponding NPOH ester was due to interfering side-chain reactions, such as formation of O-(N-carbobenzoxy-L-seryl)-N-carbobenzoxy-L-serine NPOH ester.[19]

Experimental Section[20]

General Procedure for the Preparation of N-Carbobenzoxy-L-amino Acid PCPOH Esters.—The general procedure is illustrated by the preparation of N-carbobenzoxy-L-leucine PCPOH ester. Major departures from this procedure are given below; for those compounds for which no experimental details are given, the general procedure was modified slightly, as indicated in Table I.

N-Carbobenzoxy-L-leucine PCPOH Ester.—PCPOH (13.32 g, 50 mmoles) was dissolved in ethyl acetate (200 ml) and cooled to 0°. To this solution redistilled DCC (10.32 g, 50 mmoles) in ethyl acetate (25 ml) was added and the reaction mixture stirred at 0° for 7 min. N-Carbobenzoxy-L-leucine[21] (13.25 g, 50 mmoles) in ethyl acetate (65 ml) was added and the reaction allowed to proceed at 0° for 1 hr and then for 1 hr after removal of the ice bath. N,N'-Dicyclohexylurea (DCU) was filtered off after recooling to −10°. More DCU was isolated after concentration of the filtrate and cooling at −20°. The crude ester was then precipitated from the ethyl acetate concentrate by addition of petroleum ether (bp 30–60°) and overnight refrigeration at 4°. Filtration gave 86.4% crude ester, mp 125–126.5°. Recrystallization from methanol gave the analytically pure N-carbobenzoxy-L-leucine PCPOH ester in 64% yield, mp 127–128° (lit.[22] mp 122–124°).

N-Carbobenzoxy-L-serine PCPOH Ester.—To a solution of N-carbobenzoxy-L-serine[23] (5.00 g, 20.9 mmoles) and PCPOH (16.76 g, 62.9 mmoles) in ethyl acetate (35 ml) and dimethylformamide (3.5 ml) at room temperature, DCC (4.31 g, 20.9 mmoles) was added. After 45 min at room temperature, the resulting suspension was cooled to −10°, filtered, and the filtrate washed three times with 20-ml portions of dioxane. The combined filtrate and washings were evaporated under vacuum at room temperature. The solid residue was suspended in ethyl

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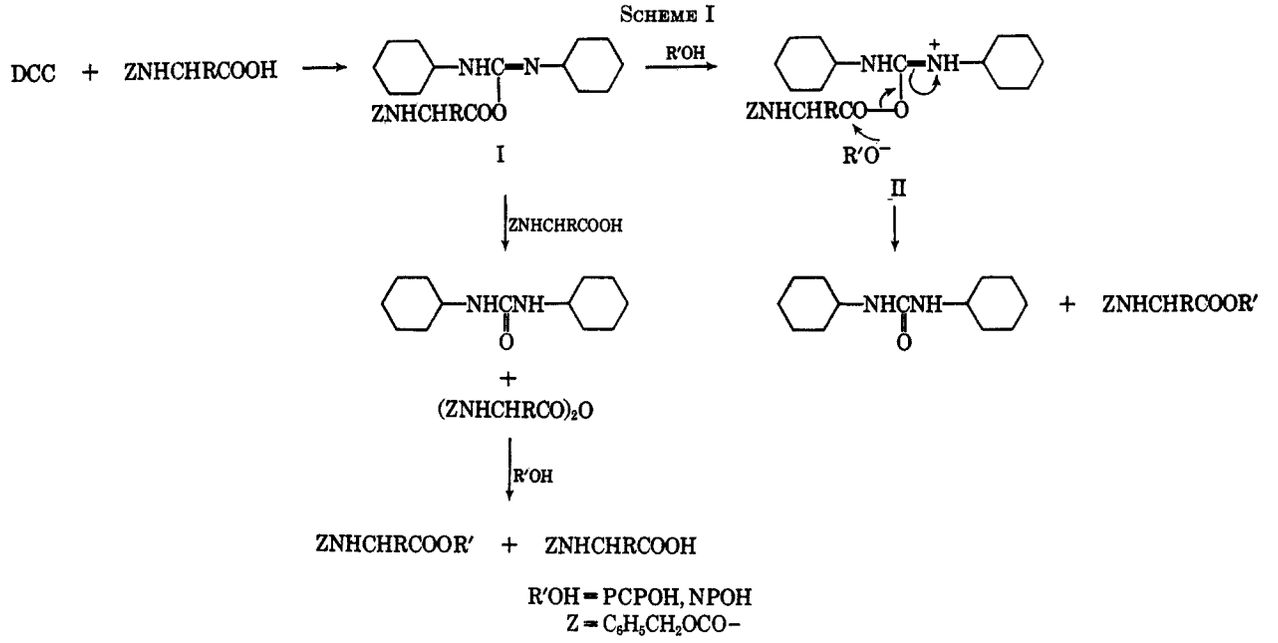
TABLE I
N-CARBOBENZOXYAMINO ACID PENTACHLOROPHENYL ESTERS

PCPOH ester[a]	Reaction conditions	Recrystn solvent[b]	Mp, °C	[α]	Formula	Calcd, % C	H	N	Cl	S	Found, % C	H	N	Cl	S
Z-Leu	*c*	MeOH	127–128[d]	$[\alpha]^{25}D$ −33.3° (*c* 0.45, MeOH)[d]	$C_{20}H_{18}NO_4Cl_5$	46.77	3.53	2.72	34.51		46.88	3.51	2.90	34.66	
Z-Val	*e*	MeOH	144–145	$[\alpha]^{27}D$ −21.0° (*c* 0.47, $CHCl_3$)	$C_{19}H_{16}NO_4Cl_5$	45.68	3.23	2.80	35.48		45.96	3.48	2.84	35.17	
Z-Met	*e*	MeOH	132–133	$[\alpha]^{26.5}D$ −1.4° (*c* 0.55, $CHCl_3$)	$C_{19}H_{16}NO_4SCl_5$	42.92	3.03	2.63	33.34	6.03	43.15	3.20	2.82	33.11	6.26
Z-Try	*e*	MeOH	167–169	$[\alpha]^{26.5}D$ −57.2° (*c* 0.90, $CHCl_3$)	$C_{25}H_{17}N_2O_4Cl_5$	51.18	2.92	4.78	30.22		51.07	3.11	4.74	29.97	
Z-Cys | Z-Cys	*f*	MeOH–DMF	215–217	$[\alpha]^{25.5}D$ −55.6° (*c* 0.63, $CHCl_3$)	$C_{34}H_{22}N_2O_6S_2Cl_{10}$	40.62	2.21	2.79	35.27	6.38	40.67	2.28	3.00	35.30	6.08
Z-Glu | NH_2	*f*	DMF–MeOH	183–187	$[\alpha]^{24.5}D$ 11.0° (*c* 0.44, DMF)	$C_{19}H_{15}N_2O_5Cl_5$	43.17	2.86	5.30			43.34	3.01	5.11		
Z-Lys | Z	*e*	MeOH	154–157	$[\alpha]^{26}D$ −7.7° (*c* 0.775, $CHCl_3$)	$C_{28}H_{25}N_2O_6Cl_5$	50.74	3.80	4.23	26.75		51.00	3.85	4.42	26.69	
Z-Ileu	*g*	MeOH–H_2O	122–124	$[\alpha]^{25.5}D$ −5.8° (*c* 0.60, $CHCl_3$)	$C_{20}H_{18}NO_4Cl_5$	46.77	3.53	2.73	34.51		47.09	3.68	2.95	34.47	
Z-Cys | BZL	*h*	DMF–MeOH	171–173	$[\alpha]^{24}D$ −34.3° (*c* 0.70, $CHCl_3$)	$C_{24}H_{18}NO_4SCl_5$	48.55	3.06	2.36	29.86	5.40	48.52	3.07	2.56	29.61	5.56
Z-γ-Abut	*e*	MeOH	134–135		$C_{18}H_{14}NO_4Cl_5$	44.52	2.91	2.88	36.51		44.80	3.12	3.00	36.78	
Z-Pro	*i*	MeOH	93–95.5	$[\alpha]^{21}D$ −47.7° (*c* 1.01, DMF)	$C_{19}H_{14}NO_4Cl_5$	45.86	2.84	2.82	35.63		46.02	2.84	2.85	35.59	
Z-Ser	*j*	1. EtOH 2. EtOAc	191.5–192.5	$[\alpha]^{25}D$ −23.6° (*c* 1.0, DMF)	$C_{17}H_{12}NO_5Cl_5$	41.88	2.48	2.87	36.36		41.88	2.43	2.87	36.51	
Z-Arg | NO_2	*k*	THF–Et_2O	109–111.5	$[\alpha]^{30}D$ −14.2° (*c* 1.01, DMF)	$C_{20}H_{18}N_5O_6Cl_5$	39.92	3.02	11.64	29.46		40.09	3.44	11.79	29.44	
Z-Glu-OBu-*t*	*l*	MeOH	122–124	$[\alpha]^{26}D$ 8.6° (*c* 3.76, CH_2Cl_2)	$C_{23}H_{22}NO_6Cl_5$	47.16	3.79	2.39	30.27		47.21	3.65	2.54	29.90	

[a] Abbreviations used here are described in Proceedings of the 5th European Peptide Symposium, Oxford, Sept 1962, G. T. Young, Ed., The Macmillan Co., New York, N. Y., 1963. [b] DMF = dimethylformamide, THF = tetrahydrofuran. [c] Ethyl acetate solution at 0° for 1 hr and then at room temperature for 1 hr. [d] Reference 1 gives mp 122–124°, $[\alpha]^{18}D$ −44.4° (*c* 0.43, methanol). However, our material, which is only 75% optically pure based on this specific rotation, gave 81% N-carbobenzoxy-L-leucylglycine ethyl ester with a specific rotation equal to, or higher than, any previous literature value (M. Q. Ceprini, Ph.D. Thesis, St. John's University, 1967). Such a yield of optically pure material is impossible if the $[\alpha]^{18}D$ −44.4° is correct. [e] Methylene chloride solution at room temperature for 3 hr. [f] Dimethylformamide solution at room temperature for 3 hr. [g] Methylene chloride solution at −10° for 3 hr and then at room temperature for 4 hr. [h] Dimethylformamide solution at −10° for 2 hr and then at room temperature for 5 hr. [i] Dimethylformamide solution at −5 to −15° for 3 hr. [j] Ethyl acetate–dimethylformamide solution at room temperature for 45 min. [k] Dimethylformamide solution at −10° for 3 hr and then at room temperature for 40 min. [l] Methylene chloride solution at room temperature for 5 hr.

SCHEME I



R'OH = PCPOH, NPOH
Z = $C_6H_5CH_2OCO-$

acetate (25 ml), diluted with petroleum ether (400 ml), and refrigerated overnight. Filtration and washing three times with 100-ml portions of petroleum ether–ethyl acetate (10:1) and then cold ether (50 ml) yielded 61.8% crude ester, mp 176–181°. Recrystallization from ethanol gave the raised mp 189–190°, yield 50%; another recrystallization from ethyl acetate gave the analytical sample, mp 191.5–192.5°.

N-Carbobenzoxynitro-L-arginine PCPOH Ester.—To a solution of N-carbobenzoxynitro-L-arginine[24] (2.0 g, 5.67 mmoles) and PCPOH (4.5 g, 17 mmoles) in dimethylformamide (10 ml), DCC (1.17 g, 5.67 mmoles) was added. Stirring was continued at room temperature for 2 hr. The mixture was then cooled to −10°, filtered, and the filtrate poured into water (300 ml). The resulting oil crystallized on trituration with water. The product was suspended in ether, diluted with petroleum ether, filtered, and washed with ether. Recrystallization from tetrahydrofuran–ether gave 2.50 g (73.5%), mp 102–105°. One more recrystallization from the same solvent gave the raised mp 109–111.5°, yield 68%. A sample was recrystallized once more for analysis.

Anal. Calcd for $C_{20}H_{18}N_5O_6Cl_5$: C, 39.92; H, 3.02; N, 11.64; Cl, 29.46. Found: C, 40.09; H, 3.44; N, 11.79; Cl, 29.44.

When a crude sample was recrystallized from dimethylformamide–ether the ester was solvated with 1 mole of dimethylformamide, mp 109.5–111°.

Anal. Calcd for $C_{20}H_{18}N_5O_6Cl_5 \cdot C_3H_7NO$: C, 40.94; H, 3.73; N, 12.46; Cl, 26.27. Found: C, 40.41; H, 3.61; N, 12.21; Cl, 26.48.

The solvated dimethylformamide was removed by two procedures: (a) trituration with water and then anhydrous ether, mp 105–110°, and (b) recrystallization from tetrahydrofuran–ether, mp 108.5–111°.

N-Carbobenzoxy-α-*t*-butyl-L-glutamate PCPOH Ester.—To a cold (0°) stirred solution of N-carbobenzoxy-α-*t*-butyl-L-glutamate[25] (7.0 g, 21 mmoles) in methylene chloride (140 ml), DCC (4.3 g, 21 mmoles) was added, followed after 5 min by PCPOH (5.6 g, 21 mmoles). Stirring was continued in the cold for 30 min and at room temperature for 5 hr. Glacial acetic acid (1 ml) was added and stirring continued for 30 min. The mixture was filtered, the precipitate (DCU) washed with methylene chloride (25 ml), and the filtrate concentrated under vacuum. The solid residue was recrystallized from methanol: yield 7.4 g (62%); mp 122–124°.

Registry No.—PCPOH ester of Z-Leu, 13758-71-9; PCPOH ester of Z-Val, 4824-13-9; PCPOH ester of Z-Met, 4841-70-7; PCPOH ester of Z-Try, 13673-49-9; PCPOH ester of Z-Cys-Cys-Z, 13673-50-2; PCPOH ester of Z-Glu-NH_2, 13673-51-3; PCPOH ester of Z-Lys-Z, 13673-52-4; PCPOH ester of Z-Ileu, 13673-53-5; PCPOH ester of Z-Cys-BZL, 13673-54-6; PCPOH ester of Z-γ-Abut, 13673-55-7; PCPOH ester of Z-Pro, 13673-56-8; PCPOH ester of Z-Ser, 13673-57-9; PCPOH ester of Z-Arg-NO_2, 5165-16-2; PCPOH ester of Z-Glu-OBu-*t*, 6233-91-6.

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Plasmalogen Synthesis. Use of 1-Alkynylglycerols and the Production of Allenic Ethers[1a]

GEORGE K. CHACKO, K. SCHILLING,[1b] AND EDWARD G. PERKINS[2]

The Burnsides Research Laboratory, Department of Food Science, University of Illinois, Urbana, Illinois 61801

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Attempts to synthesize plasmalogens have been recently reported from several laboratories.[3-8] An apparently convenient synthetic route to 1-alkenyl ethers of isopropylideneglycerol through the corresponding acetylenic ethers as intermediates was re-

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(2) Author to whom reprint requests should be addressed.

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