t-Amyloxycarbonyl as a New Protecting Group in Peptide Synthesis. I. The Synthesis and Properties of *N*-*t*-Amyloxycarbonylamino Acids and Related Compounds

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The *t*-butoxycarbonyl group (BOC group) is well known as a useful *N*-protecting group in the preparation of complicated peptides. Although *t*-butyl azidoformate,[1] *t*-butyl *p*-nitrophenyl carbonate[2] and *t*-butyl cyanoformate[3] have been recommended as *t*-butoxycarbonylating reagents, these can not be synthesized as easily as carbobenzoxy chloride. Thus, there is as yet no good practical method for synthesizing BOC-amino acids on a large scale. If *t*-butyl chloroformate, which corresponds to carbobenzoxy chloride, were stable against water or amines under normal conditions, it would be a most useful reagent for the purpose.[2,4]

In the present investigation, the *t*-amyloxycarbonyl group (AOC group) was found to be a much more convenient *N*-protecting group than BOC in many cases. The AOC group could be introduced quite readily into amino acid esters by using *t*-amyl chloroformate (I) as a reagent. Reagent I was originally prepared by Merck's group in 1912 as a material for hypnotics.[5] According to the description of the patent, it was synthesized by adding quinoline to a cold mixture of *t*-amyl alcohol and phosgene. When this procedure was tested, however, it was found that the product was colored and decomposed spontaneously within few days, even in a deep freezer at −20°C. Therefore, the procedure was improved in the present study. It was found that pyridine was a better condensing reagent than quinoline, and that excess phosgene was necessary to obtain a good yield of reagent I. The reagent thus obtained was a colorless liquid, which did not decompose when stored for over ten days in a deep freezer. The *N*-acylation reaction with amino acid esters was carried out readily in chloroform in the presence of triethylamine, with an almost quantitative yield. The AOC-esters were then converted to their respective free acids or hydrazides by the normal procedure. Generally, the hydrogenation of benzyl esters gave better yields of free acids than the saponification of ethyl or methyl esters. The yields, physical constants and analytical data of the AOC-amino acids are listed in Table I.

Since reagent I was unstable in the presence of water, it was not possible to get AOC-amino

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2) G. W. Anderson and A. C. McGregor, *J. Am. Chem. Soc.*, **79**, 6180 (1957).

3) L. A. Carpino, ibid., **82**, 2725 (1960).

4) A. R. Choppin and J. W. Rogers, ibid., **70**, 2967 (1948).

5) Merck, German. Pat. 254471 (1912); *Chem. Zentr.*, **1913 I**, 346.

TABLE I. YIELDS AND PROPERTIES OF *N*-*t*-AMYLOXYCARBONYLAMINO ACIDS

AOC-deriv. of	Parent ester	Recryst. solvent	Yield, %	M. p., °C	$[\alpha]_D^{21}$ (*c* in EtOH)
L-Alanine	Benzyl		91	Oil	
β-Methyl-L-aspartate	*p*-Nitrobenzyl		72	Oil	
γ-Methyl-L-glutamate	*p*-Nitrobenzyl[a]		80	Oil	
Glycine[b]	Ethyl	AcOEt - Pet. ether	82	82.5—84	
L-Isoleucine	Methyl		73	Oil	
L-Isoleucine	Benzyl		100	Oil	
L-Leucine[c]	Ethyl	EtOH-Water	79	61—63	−18.6°(1.9)
L-Lysine (di-AOC-deriv.)	Benzyl		96	Oil	
α-Carbobenzoxy L-lysine[d]	Methyl		98	Oil	
L-Methionine	Methyl		76	Oil	
δ-Carbobenzoxy L-ornithine	Methyl		87	Oil	
L-Phenylalanine	Ethyl		97	Oil	
D-Phenylalanine	Methyl		93	Oil	
L-Proline[e]	Benzyl	AcOEt - Pet. ether	90	94—95.5	−47.2°(1.7)
L-Serine	Methyl		98	Oil	
L-Tryptophan[f]	Methyl	AcOEt - Pet. ether	86	121—123	+7.1°(1.2)
L-Tyrosine	Methyl		67	Oil	
L-Valine	Ethyl		69	Oil	
L-Valine	Benzyl		94	Oil	

a) *N*-AOC-γ-methyl-α-*p*-nitrobenzyl-L-glutamate was crystallized; m. p. 66—67°C, $[\alpha]_D^{22}$ −21.9° (*c* 2, EtOH). Found: C, 55.60; H, 6.39; N, 6.83. Calcd. for $C_{19}H_{26}O_8N_2$; C, 55.68; H, 6.24; N, 6.80%. b) Found: C, 50.91; H, 8.10; N, 7.42. Calcd. for $C_8H_{15}O_4N$: C, 50.78; H, 7.99; N, 7.40%. c) Crystallized as monohydrate. Found: C, 55.08; H, 9.48; N, 5.42. Calcd. for $C_{12}H_{23}O_4N \cdot H_2O$: C, 54.72; H, 9.57; N, 5.31%. d) cf. B. Bezas and L. Zervas, *J. Am. Chem. Soc.*, **83**, 719 (1961). e) Found: C, 57.57; H, 8.21; N, 5.97. Calcd. for $C_{11}H_{19}O_4N$: C, 57.62; H, 8.35; N, 6.11%. f) Found: C, 64.68; H, 7.14; N, 8.59. Calcd. for $C_{17}H_{22}O_4N_2$: C, 64.13; H, 6.97; N, 8.80%.

acids directly by the Schotten-Baumann procedure; this was a disadvantage of the method. Some AOC-amino acids were prepared as oils, but usually good crystalline derivatives could be derived from them, as with BOC-derivatives. These crystalline derivatives are listed in Table II. When the cleavage of the AOC group from various derivatives was tested, it was found that trifluoroacetic acid or anhydrous hydrogen chloride in an organic solvent decomposed AOC readily at room temperature. This property was quite similar to that of the corresponding BOC-derivatives in all cases. Since it is possible to prepare several moles of reagent I within 24 hr., this procedure should be of great use in the practical synthesis of complicated peptides.

Incidentally, 3-methyl-3-pentyl chloroformate (II) was synthesized, and several kinds of 3-methyl-3-pentoxycarbonylamino acids (MPC-acids) were prepared by following the same procedure as was used in the case of AOC-amino acids. These data are listed in Table III. Although the MPC group could be removed, as in the case of the AOC group, the difficulty of obtaining 3-methyl-3-pentanol would limit the application of the procedure.

Experimental

***t*-Amyl Chloroformate (I).**—Dried phosgene (105 g., 1.06 mol.) was introduced into a solution of *t*-amyl alcohol (46 g., 0.52 mol.) in dry ether (500 ml.), and then the mixture was cooled to −60°C in an acetone - dry ice bath. A solution of pyridine (41 g., 0.52 mol.) in dry ether (500 ml.) was added dropwise into the cooled mixture with vigorous stirring. Then the reaction mixture was stored overnight in a deep freezer at −20°C. The pyridine hydrochloride which formed was removed by filtration, and the mother liquor was concentrated to a small volume (about 120 ml.) in an ice water bath under reduced pressure. The product thus obtained was used without further purification in the following reactions. Its purity was determined by acylating a known amount of phenylalanine methyl ester as described below; it was found that about 4 ml. of the product corresponded to 0.01 mol. The yield was about 60%.

3-Methyl-3-pentyl Chloroformate (II).—When 3-methyl-3-pentanol (b. p. 119—122°C; 31 g., 0.3 mol.) was allowed to react with phosgene (99 g., 1 mol.) as in the case of I using pyridine (24 g., 0.3 mol.), crude II was obtained as a colorless liquid (about 135 ml.). About 15 ml. of the product corresponded to 0.01 mol. The yield was about 30%.

TABLE II. CRYSTALLINE DERIVATIVES OF *N*-*t*-AMYLOXYCARBONYLAMINO ACIDS

Compound	Recryst. solvent	M. p., °C	Yield %	$[\alpha]_D^{21}$ (*c* in Ethanol)	Empirical formula	Anal. Calcd. / Found. C %	H %	N %
A) Dicyclohexylamine salt of AOC-deriv. of								
L-Alanine	Ether-Pet. ether	124—126	78	+4.7°(2.4)[a]	$C_{21}H_{40}O_4N_2$	65.59 65.88	10.48 10.45	7.29 7.40
β-Methyl-L-aspartate	AcOEt-Pet. ether	123—124	91	+19.4°(1.93)[b]	$C_{23}H_{42}O_6N_2$	62.34 62.41	9.55 9.57	6.38 6.33
γ-Methyl-L-glutamate	AcOEt-Pet. ether	133—134	70	+13.5°(1.97)[b]	$C_{24}H_{44}O_6N_2$	63.32 63.13	9.61 9.71	6.09 6.14
L-Isoleucine	Ether-Pet. ether	101.5—102	71	+3.9°(1.6)[a]	$C_{24}H_{46}O_4N_2$	67.56 67.65	10.87 10.89	6.57 6.76
α-Carbobenzoxy-L-lysine	AcOEt		80	+5.54°(3.1)[a]	$C_{32}H_{53}O_6N_3$	66.75 66.82	9.28 8.90	7.30 7.18
L-Methionine	AcOEt-Pet. ether	105—107	75	+17.4°(1.3)	$C_{23}H_{44}O_4N_2S$	62.12 61.96	9.97 9.82	6.03[d] 6.17
L-Phenylalanine	AcOEt	198—199	85	+27.4°(0.79)	$C_{27}H_{44}O_4N_2$	70.40 70.15	9.63 9.48	6.08 5.97
L-Tyrosine	AcOEt-MeOH	203—204	78	+44.3°(1.0)[a]	$C_{27}H_{44}O_5N_2$	68.03 67.89	9.31 9.23	5.88 5.89
L-Valine	Ether-Pet. ether	119—121.5	79	+2.0°(1.7)	$C_{23}H_{44}O_4N_2$	66.95 66.61	10.75 10.75	6.79 6.72
B) *p*-Nitrophenyl ester of AOC-deriv. of								
L-Leucine	Ether-Pet. ether	59—61	79	−49.3°(1.3)	$C_{18}H_{26}O_6N_2$	59.00 58.96	7.15 7.08	7.65 7.62
L-Lysine (di-AOC deriv.)	Ether-Pet. ether	65—68	46	−25.2°(1.8)	$C_{24}H_{37}O_8N_3$	58.16 58.43	7.53 7.41	8.49 8.40
δ-Carbobenzoxy-L-ornithine	AcOEt-Pet. ether	110—112	50	−27.5°(2.3)[c]	$C_{25}H_{31}O_8N_3$	59.87 59.83	6.23 6.32	8.38 8.56
L-Phenylalanine	AcOEt-Pet. ether	128—129	83	−14.6°(1.3)	$C_{21}H_{24}O_6N_2$	62.99 63.02	6.04 6.07	7.00 6.92
D-Phenylalanine	AcOEt-Pet. ether	128—128.5	81	+15.4°(1.0)	$C_{21}H_{24}O_6N_2$	62.99 63.00	6.04 6.08	7.00 7.05
C) AOC-L-serine hydrazide	AcOEt	98—100	82	−5.0°(1.0)	$C_9H_{19}O_4N_3$	46.34 46.32	8.21 8.33	18.02 17.98

a) 22°C. b) 20°C. c) 24°C. d) Calcd.: S, 7.21. Found: S, 7.04%.

AOC- and MPC-amino Acids.—Into a solution of an amino acid-ester hydrochloride or tosylate (0.1 mol.) in chloroform (200 ml.), portions of I or II and triethylamine (0.1 mol.) were added alternately at −5 to −10°C. The addition of I or II was continued untill the ninhydrin-positive material had disappeared from the reaction mixture; this was checked by thin-layer chromatography. Then, the reaction mixture was washed successively with water, 0.5 N hydrochloric acid and a 5% sodium bicarbonate solution. The solution was dried over anhydrous magnesium sulfate and then concentrated to an oil. The yied of the oil (AOC-ester) was generally quantitative. Then the esters were subjected to saponification in acetone or, if benzyl esters, to hydrogenation in a suitable organic solvent (such as methanol); the AOC- or MPC-amino acids were obtained after following the usual purification procedures (Tables I and III).

AOC-L-serine Hydrazide.—AOC-L-serine methyl ester, which was obtained from L-serine methyl ester hydrochloride (3.9 g., 0.025 mol.), was dissolved in ethanol (18 ml.), and then hydrazine hydrate (4 ml.) was added to the solution. After the solution had stood for about 20 hr. at room temperature, it was concentrated to dryness, and the residue was recrystallized from ethyl acetate (Table II).

Dicyclohexylamine Salts of AOC- and MPC-amino Acids.—Each AOC- or MPC-amino acid was mixed with an equimolar amount of dicyclohexylamine in dry ether. The crystals which appeared were filtered off and recrystallized from a suitable solvent system. When the product was soluble in ether, it was precipitated by the addition of petroleum ether to the solution. The data are listed in Tables II and III.

AOC-amino Acid *p*-Nitrophenyl Ester.—A solution of each AOC-amino acid and an equimolar amount of *p*-nitrophenol in ethyl acetate was treated with an equimolar amount of dicyclohexylcarbodiimide at −10°C for one hour, and then the mixture was allowed to react for an additional 2 hr. at room temperature. The dicyclohexylurea which formed was removed by filtration, and the mother liquor was concentrated to dryness. The residual product was recrystallized from a suitable solvent system. The data are listed in Table II.

TABLE III. 3-METHYL-3-PENTOXYCARBONYLAMINO ACIDS AND THEIR DICYCLOHEXYLAMINE SALTS

Compound	Recryst. solvent	M. p., °C	Yield %	$[\alpha]_D^{24}$ (*c* in Ethanol)	Empirical formula	Anal. Calcd. / Found		
						C	H	N
						%	%	%
A) MPC-deriv. of								
Glycine	AcOEt-Pet. ether	71.5—73	84		$C_9H_7O_4N$	53.19 53.02	8.43 8.42	6.89 7.00
L-Leucine		Oil	84					
L-Proline		Oil	90					
B) Dicyclohexylamine salt of MPC-deriv. of								
L-Leucine	Pet. ether	114—116.5	63	−4°(2)	$C_{25}H_{48}O_4N_2$	68.14 68.17	10.98 10.91	6.36 6.55
L-Proline	AcOEt-Pet. ether	127.5—128.5	77	−29.5°C(2)	$C_{24}H_{44}O_4N_2$	67.88 67.56	10.45 10.22	6.60 6.51

The Cleavage of the AOC Group from AOC-glycine.—a) AOC-glycine (1.14 g., 0.006 mol.) was dissolved in trifluoroacetic acid (3 ml.). After the solution had been kept at room temperature for 40 min., it was concentrated to dryness. The residue was dissolved in ethanol (10 ml.), and the solution was neutralized with triethylamine. The crystals which appeared were collected by filtration and washed well with ethanol. The recrystallization of the product from water-ethanol afforded glycine (0.42 g., 93% yield), which was identified with an authentic sample by infrared spectral analysis.

b) AOC-glycine (1.90 g., 0.01 mol.) was dissolved in 10% anhydrous hydrogen chloride in acetic acid (4 ml.). After about 30 min. ether (20 ml.) was added to the reaction mixture in order to precipitate the product completely, and the latter was collected by filtration. Then the crystals were dissolved in ethanol (25 ml.), and the solution was neutralized with pyridine (about 1.2 ml.) to precipitate free glycine. The product (glycine) was collected by filtration, washed well with ethanol, and then recrystallized from water-ethanol; the yield of glycine was 97% (0.730 g.).

Summary

It has been shown that the *t*-amyloxycarbonyl group (AOC group) can be introduced quite readily into amino acid esters by using *t*-amyl chloroformate as a reagent, and that the AOC group can be cleaved as smoothly as the *t*-butoxycarbonyl group (BOC group) under acidic conditions. Since this reagent is much easier to prepare than any other reagents used for the introduction of the BOC group, the new procedure is considered to be of great use in the practical synthesis of complicated peptides. 3-Methyl-3-pentoxycarbonylamino acids were also prepared by following the same procedure as was used in the case of AOC-amino acids.

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