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t-Amyloxycarbonyl as a New Protecting Group in Peptide Synthesis. V. Direct Synthesis of *t*-Amyloxycarbonyl- and *t*-Butyloxycarbonyl-amino Acids Using the Respective *t*-Alkyl Chloroformates

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t-Amyloxycarbonylamino acids (Aoc-amino acids) were found to be synthesizable under the conditions of the Schotten-Baumann reaction with *t*-amyl chloroformate in the presence of a water-soluble inert solvent. The procedure was applied to almost all amino acids or their derivatives; the yields of the products and their purities were satisfactory except in the cases of asparagine, glutamine, and nitroarginine. Many *t*-butyloxycarbonylamino acids (Boc-amino acids) were also synthesized successfully using the same procedure. Similarly, *t*-amyl and *t*-butyl carbazates were synthesized in satisfactory yields with these chloroformates and hydrazine hydrate. Thus, the procedure was demonstrated to be the simplest for the preparation of Aoc- or Boc-amino acids and hydrazide.

In this series of studies,[1-4] it has previously been established that *t*-amyl chloroformate (I) is a useful reagent for introducing the Aoc-group into amino acid esters in an organic solvent, and that Aoc-amino acids can be obtained conveniently by the removal of the ester groups. *t*-Amyl carbazate has also been synthesized by the condensation of reagent I with anhydrous hydrazine, and many Aoc-amino acids have been prepared with the carbazate as in the case of the standard method for the synthesis of Boc-amino acids. The Aoc-group thus introduced can be removed during peptide synthesis, as in the case of the Boc-group. The Aoc-method has already been applied to the syntheses of many peptides.[2,5-10]

As was mentioned in the first paper,[1] the introduction of the Aoc-group into free amino acid was unsuccessful with the reagent I when the reaction was carried out under the standard conditions of the Schotten-Baumann reaction. This was attributed to the instability of the reagent I in water. During repeated trials, however, it was found that the addition of an inert water-soluble organic solvent, such as tetrahydrofuran, isopropanol, or methanol, increased the rate of the acylation reaction in comparison with that of the decomposition of I. Finally, new practical conditions were established for the direct preparation of the Aoc-amino acids with the reagent I by the Schotten-Baumann reaction. The reagent I which was synthesized by the ether procedure[1] was preferable for these reactions to that prepared by the toluene method,[4] because the presence of a water-insoluble solvent in the Schotten-Baumann reaction mixture tended to increase the formation of uncertain by-products. Although this procedure was satisfactory for almost all amino acids and their side-chain derivatives, low yields and unsatisfactory purities were observed in the syntheses of Aoc-asparagine, -glutamine, and -nitroarginine, as is shown in Table 1. These phenomena may account for the partial formation of their mixed anhydrides during the reaction, which in these cases may be further transformed to the nitriles or lactams.

The synthesis of *t*-amyl carbazate[4] has been

1) S. Sakakibara, M. Shin, M. Fujino, Y. Shimonishi, S. Inouye and N. Inukai, This Bulletin, **38**, 1522 (1965).
2) S. Sakakibara and M. Fujino, *ibid.*, **39**, 947 (1966).
3) S. Sakakibara and M. Itoh, *ibid.*, **40**, 646 (1967).
4) I. Honda, Y. Shimonishi and S. Sakakibara, *ibid.*, **40**, 2415 (1967).
5) S. Sakakibara and N. Inukai, *ibid.*, **39**, 1567 (1966).
6) S. Sakakibara, Y. Shimonishi, M. Okada and Y. Kishida, "Peptides," Proc. 8th European Peptide Symp. (1966), edited by H. C. Beyermann *et al.*, North-Holland, Amsterdam (1967), p. 44.
7) N. Inukai, K. Nakano and M. Murakami, This Bulletin, **40**, 2913 (1967).
8) N. Inukai, K. Nakano and M. Murakami, *ibid.*, **41**, 182 (1968).
9) S. Sakakibara, Y. Kishida, R. Nishizawa and Y. Shimonishi, *ibid.*, **41**, 438 (1968).
10) S. Sakakibara, Y. Kishida, Y. Kikuchi, R. Sakai and K. Kakiuchi, *ibid.*, **41**, 1273 (1968).

TABLE 1. YIELD AND PHYSICAL CONSTANTS OF AOC-AMINO ACIDS SYNTHESIZED BY THE DIRECT METHOD

Aoc-amino acid	Melting point °C observed	cited[a]	Yield[b] %	$[\alpha]_D$ observed	cited[a]	Temp °C
Gly	84—85.5		74			
		84—85.5				
L-Leu[c]	66—68		75	−17.8° (*c* 2, EtOH)		20
		66—68		−17.3° (*c* 2, EtOH)		20
L-Pro	98—99		88	−47.6° (*c* 1.8, EtOH)		25
		98—99		−47.6° (*c* 2, EtOH)		20
L-Phe[d]	208—210 (decomp.)		77	+38.2° (*c* 0.52, EtOH)		26
		209—211		+40.6° (*c* 0.67, EtOH)		20
L-Asp(NH_2)[e]	151—152 (decomp.)		45	−7.6° (*c* 3.7, DMF)		25
		151.5—152.5		−8.2° (*c* 3.4, DMF)		28
L-Glu(NH_2)[e]	116.5—117.5		36	−17.6° (*c* 3.9, DMF)		25
		115.5—116.5		−19.4° (*c* 4.8, DMF)		20
L-Arg(NO_2)	139 (decomp.)		44	−25.5° (*c* 2.1, pyridine)		25
		139—141 (decomp.)		−28.2° (*c* 2, pyridine)		18
L-Tyr(Bzl)[d]	114—116		85	+42.6° (*c* 4.8, EtOH)		21
		113.5—115.5		+43.1° (*c* 5, EtOH)		21

a) The same lit. as in Ref. 4.
b) Calcd from amino acid after recystallization.
c) Obtained as crystals of mono-hydrate; optical rotation calcd for the hydrate.
d) Obtained as crystals of the dicyclohexylamine salt.
e) Isopropanol (300 m*l*), in stead of methanol, and 3 equivalents of reagent I were used for the reaction.

TABLE 2. YIELD AND PHYSICAL CONSTANTS OF BOC-AMINO ACIDS SYNTHESIZED BY THE DIRECT METHOD

Boc-amino acid	Melting point °C observed	cited	Yield[a] %	$[\alpha]_D$ observed	cited	Temp. °C
Gly	88—89		74			
		88.5—89[b]				
L-Leu[c]	82—83		89	−23.9° (*c* 1.4, AcOH)		25
		67—72[b]		−24.0° (*c* 2, AcOH)[b]		25
L-Phe[d,e]	221—223 (decomp.)		73	−1.7° (*c* 1, AcOH)		25
L-Pro	133—135		90	−60.4° (*c* 2.2, AcOH)		25
		136—137[b]		−60.2° (*c* 2, AcOH)[b]		25
L-Lys(Z)[d]	111—113		66	−7.2° (*c* 1.1, AcOH)		25
		110—111[f]		−9.3° (*c* 1, AcOH)[g,f]		18—25
L-Met[d]	137—139		56	+12.3° (*c* 2.1, MeOH)		25
		138—139[f]		+19.0° (*c* 1, MeOH)[g,f]		18—25

a) Calcd from amino acid after recrystallization.
b) The same lit. as in Ref. 11.
c) Obtained as crystals of mono-hydrate.
d) Obtained as crystals of the dicyclohexylamine salt.
e) Found: C, 69.69; H, 9.85; N, 6.17%. Calcd for $C_{26}H_{42}O_4N_2$: C, 69.92; H, 9.48; N, 6.27%.
f) E. Schnabel, *Ann.*, **702**, 188 (1967).
g) Observed at 578 mμ.

simplified further in the present study; that is, commercially-available hydrazine hydrate (80—90%) was found to be a satisfactory material when the reagent I was slowly added to it for the coupling reaction. The yield of *t*-amyl carbazate was almost the same as that previously reported with anhydrous hydrazine.[4]

The same technique was also applied to the synthesis of Boc-amino acids and of the carbazate. Although Anderson and McGregor[11] abandoned the use of *t*-butyl chloroformate (II)[12] as a reagent for the preparation of Boc-amino acids, these Boc-derivatives were found to be synthesized similarly,

11) G. W. Anderson and A. C. McGregor, *J. Am. Chem. Soc.*, **79**, 6180 (1957).
12) A. R. Choppin and J. W. Rogers, *ibid.*, **70**, 2967 (1948).

and in almost the same yields, as Aoc-derivatives (see Table 2).

One big difference between Aoc- and Boc-derivatives was in their solubilities in water. Although there was no difficulty in extracting Aoc-amino acids or hydrazide from the aqueous reaction media with ether, a strong salting-out was usually necessary to extract the Boc-amino acids or hydrazide from the aqueous reaction mixture. This difference should also be kept in mind when Aoc- or Boc-peptide derivatives are synthesized.

Experimental

All the reactions with the reagent I or the reagent II were monitored by thin-layer chromatography using Silica-gel G; the solvent system was chloroform : methanol : acetic acid (95 : 5 : 3 v/v) or *n*-butanol : acetic acid : water (4 : 1 : 1 v/v). After separation, the spots were located with ninhydrin at 100°C or with iodine vapor at room temperature.

***t*-Amyl Chloroformate (I).** Liquid phosgene was collected and measured (200 m*l*, *ca.* 2.8 mol) in a three-necked flask (capacity 5 *l*), and then a solution of *t*-amyl alcohol (176 g, 2 mol) in dry ether (3 *l*) was added to it. A solution of dry pyridine (150 g, 1.9 mol) in dry ether (1 *l*) was added slowly to the mixture at −20——30°C over a period of 90 min, taking precautions to prevent the introduction of moisture. Efficient stirring was necessary in order to obtain a homogeneous reaction. Stirring was continued for a further 30 min at the same temperature, and then the mixture was kept overnight in a deep freezer at −20°C. The precipitate thus formed was filtered off, again taking care to prevent the introduction of moisture; the filtrate was then concentrated to about 400 m*l* under reduced pressure in an ice-water bath. This solution was used as the stock solution of I without further purification. (It should be kept dry in a deep freezer and use within 10 days.)

***t*-Butyl Chloroformate (II).** Essentially the same procedure was followed with *t*-butyl alcohol (148 g, 2 mol). The final solution was used as the stock solution of II without further purification.

***t*-Amyloxycarbonyl-L-proline** (General Procedure for the Synthesis of Aoc-amino Acids). A stock solution of I (1 mol, calcd from *t*-amyl alcohol) was added slowly to a solution of L-proline (69 g, 0.6 mol) in a mixture of methanol (150 m*l*) and 2 N aqueous sodium hydroxide (600 m*l*) at 0——5°C. The mixture was agitated vigorously with a mechanical stirrer during the addition of I (over about one hour). The stirring was continued for a further 2 hr at 0°C, and then for an additional hour at room temperature. (During the reaction, the solution should be kept above pH 8 by the addition of 4 N aqueous sodium hydroxide. After the addition of I, the extent of the reaction should be checked by thin-layer chromatography; if appreciable proline remains, more reagent should be added.) Then, the reaction mixture was adjusted to pH 2—3 with 1 N hydrochloric acid, and the product was extracted with ethyl acetate (300 m*l*×1, 100 m*l*×3). The combined extract was dried over sodium sulfate. On the concentration of the dried solution, crystals were obtained which were then recrystallized from ethyl acetate and petroleum ether; yield 137 g (88%). The physical constants of the material are shown in Table 1.

***t*-Amyl Carbazate.** Reagent I (1 mol, calcd from *t*-amyl alcohol) was added, over a 3-hr period, to a suspension of 80% hydrazine hydrate (250 g, 4 mol) in ether (1 *l*) at 0——3°C. The slow addition of I and a vigorous stirring of the reaction mixture were essential to obtain a good yield. Stirring was continued at 0°C for an additional 2 hr after the complete addition of I. Then, 4 N sodium hydroxide (250 m*l*) was added to the reaction mixture, the ether layer was separated, and the aqueous layer was extracted with ether (100 m*l*×3). The ether layer and the extracts were combined, washed with a saturated solution of sodium chloride, and dried over sodium sulfate. The removal of ether from the dried solution left an oily material, which was then purified by fractional distillation under reduced pressure; yield, 83 g (57%, calcd from *t*-amyl alcohol), bp 85—86°C/4—5 mmHg. This value was essentially the same as that reported previously.[4]

***t*-Butyloxycarbonylglycine** (General Procedure for the Synthesis of Boc-amino Acids). Glycine (45 g, 0.6 mol) was dissolved in a mixture of methanol (120 m*l*) and 2 N aqueous sodium hydroxide (600 m*l*). The solution was treated with II (1 mol, calcd from *t*-butyl alcohol) following the procedure used for the synthesis of Aoc-L-proline with I. The reaction mixture was acidified to pH 2—3 with 1 N aqueous hydrochloric acid and saturated with sodium chloride; then the product was extracted with ethyl acetate (200 m*l*×2, 100 m*l*×5). The combined extract was washed with a saturated solution of sodium chloride (50 m*l*×2) and dried over sodium sulfate. The concentration of the dried solution left crystals which were then recrystallized from ethyl acetate and petroleum ether; yield, 77 g (74%). The melting point of this compound is shown in Table 2.

***t*-Butyl Carbazate.** The procedure used for the synthesis of *t*-amyl carbazate was followed with the reagent II (1 mol, calcd from *t*-butyl alcohol). The reaction product was extracted with ether (200 m*l*×2, 100 m*l*×5) after the addition of 4 N sodium hydroxide (250 m*l*) and saturation with sodium chloride. The combined ether extract was dried over sodium sulfate, and the dried solution was concentrated to a residue which was purified by fractional distillation; yield, 77 g (58%), bp 92—93°C/10 mmHg, mp 39.5—41.5°C. Reported, bp 120—122°C/15 mmHg, mp 41—42°C.[13]

13) Y. A. Ovchinnikov, A. A. Kiryushkin and A. I. Miroshnikov, *Experientia*, **21**, 418 (1965).