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t-Butyloxycarbonylamino Acids and Their Use in Peptide Synthesis

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The preparation of a number of *t*-butyloxycarbonylamino acids by the use of a new reagent, *t*-butyl *p*-nitrophenyl carbonate, is described. These amino acid derivatives compare favorably with benzyloxycarbonylamino acids (carbobenzoxyamino acids) in physical properties. Their use in the synthesis of peptides is described. The *t*-butyloxycarbonyl group is readily removed by hydrogen halides, and is resistant to hydrogenation and to sodium in liquid ammonia. The synthesis and use in peptide-bond formation of a new pyrophosphite reagent, diethyl ethylenepyrophosphite, is described.

The introduction of the benzyloxycarbonyl (carbobenzoxy) group for the masking of amine groups[1] was a major contribution to peptide syntheses. Benzyloxycarbonyl derivatives are readily made and are usually crystalline, and the protecting group can be removed by several procedures. A number of analogous aryl- or alkyloxycarbonyl protecting groups have been investigated.[2] None of these provided the modification of the properties of the carbobenzoxy group of interest to us: increased ease of removal under mild conditions with the formation of gaseous or low-boiling by-products.

The carbobenzoxy group is most conveniently removed from peptide derivatives by treatment with hydrogen bromide in glacial acetic acid.[3-5] By-products of this reaction are carbon dioxide and benzyl bromide. Alkyloxycarbonyl groups analogous to carbobenzoxy might be expected to give carbon dioxide and alkyl bromides, and the latter would be much more volatile than benzyl bromide. However, it has been reported[2] that the reactivity of ethyloxycarbonylamino- and *sec*-butyloxycarbonylamino acids and peptides to hydrogen bromide in acetic acid is considerably less than that of the corresponding carbobenzoxy derivatives. The *t*-butyloxycarbonylamino derivatives were not reported; these are especially interesting because of the possibility of formation of the gas isobutylene.

$$(CH_3)_3COCNHR \xrightarrow{H^+} (CH_3)_2C{=}CH_2 + CO_2 + NH_2R$$

Exploratory reactions with the known[6,9] *t*-butyl N-phenylcarbamate demonstrated that aniline hydrochloride was formed by warming with a solution of hydrogen chloride in benzene or in diethyl phosphite. In the latter solvent, an equivalent of hydrogen chloride gave a 56% yield of aniline hydrochloride, and two equivalents of hydrogen chloride gave an 89% yield. This is indicative that *t*-butyl chloride might have been the by-product instead of isobutylene.[7] Further work will be necessary to prove the nature of the by-product. Benzyl N-phenylcarbamate[8] has been found to be unreactive to hydrogen chloride in diethyl phosphite under the same conditions.

The synthesis of *t*-butyloxycarbonylamino acids (*t*-BOC-amino acids) by the use of *t*-butyl chloroformate, analogous to the synthesis of benzyloxycarbonylamino acids from benzyl chloroformate, was found to be impractical. Choppin and Rogers[9] reported that *t*-butyl chloroformate, prepared from phosgene and sodium *t*-butoxide at low temperatures, is an unstable compound which begins to decompose at 10°. Numerous attempts on our part to use this compound were unsatisfactory; even at low temperatures low yields of the carbamates of aniline and methyl glycinate were obtained.

The use of isocyanates derived from amino acid esters by reaction with phosgene[10] provided an indirect route to *t*-BOC-amino acids. Reaction of the isocyanate derived from methyl glycinate with *t*-butyl alcohol in the presence of triethylamine[11] yielded methyl *t*-BOC-glycinate as an oil which was then saponified to give crystalline *t*-BOC-glycine. This procedure is generally unattractive, however, because of the number of steps in the synthesis, and complications such as the formation of urea by-products in the *t*-butyl alcohol–isocyanate reaction.

Another approach was found in the use of phenyl *t*-butyl carbonate. The carbamates of a number of tertiary acetylenic carbinols have been synthesized by reaction of ammonia with the corresponding phenyl carbonates[12] and recently the synthesis of *t*-butyl phenyl carbonate and its reaction with hydrazine to form *t*-butyl carbazate has been reported.[7] Phenyl *t*-butyl carbonate has been prepared by us with some difficulty; Carpino[7] indicates that exacting conditions are necessary. It appeared to us that *t*-butyl *p*-nitrophenyl carbonate might have advantages in ease of preparation and in increased reactivity. In both aspects it has proved to be a satisfactory reagent. It is readily prepared in good yield by the reaction of *p*-nitrophenyl chloroformate[13] with *t*-butyl al-

(1) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

(2) *Cf.* R. A. Boissonnas and G. Preitner, *Helv. Chim. Acta*, **36**, 875 (1953).

(3) G. W. Anderson, J. Blodinger and A. D. Welcher, THIS JOURNAL, **74**, 5311 (1952).

(4) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).

(5) R. A. Boissonnas and G. Preitner, *Helv. Chim. Acta*, **35**, 2240 (1952).

(6) E. Knoevenagel, *Ann.*, **297**, 148 (1897).

(7) In an investigation independent of the work reported here, L. A. Carpino (THIS JOURNAL, **79**, 98–101 (1957)) recently has reported the cleavage of *t*-butyl N-phenylcarbamate by acids under mild conditions and has suggested that the *t*-butyloxycarbonyl group will be generally useful as a protecting group for amines. He gave indirect evidence for the formation of isobutylene in the cleavage reaction.

(8) D. Ben-Ishai and E. Katchalski, *J. Org. Chem.*, **16**, 1025 (1951).

(9) A. R. Choppin and J. W. Rogers, THIS JOURNAL, **70**, 2967 (1948).

(10) S. Goldschmidt and M. Wick, *Ann.*, **575**, 217 (1952).

(11) J. W. Baker and J. Gaunt, *J. Chem. Soc.*, 9 (1949).

(12) W. M. McLamore, S. Y. Pan and A. Bavley, *J. Org. Chem.*, **20**, 1379 (1955).

(13) German Patent 287,805; *Friedl.*, **12**, 694 (1915).

TABLE I: PROPERTIES OF *t*-BUTYLOXYCARBONYLAMINO ACIDS

t-Butyloxycarbonyl derivative of	Method and base used	Recrystallization solvent	Yield,[a] %	M.p., °C.	Neut. equiv. Calcd.	Neut. equiv. Found	$[\alpha]^{25}D$ in glacial acetic acid	Empirical formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N
DL-Alanine	A NaOH	Ether–petr. ether	62	110.5–111.5	189	196		$C_8H_{15}O_4N$	50.78	7.99	7.40	50.84	8.21	7.52
L-Alanine	A NaOH	Ether–petr. ether	55.5	83–84	189	193	−22.4° (2.095%)	$C_8H_{15}O_4N$	50.78	7.99	7.40	50.77	8.17	7.09
S-Benzyl-L-cysteine	B $NaHCO_3$	Ether–petr. ether	16.5	63–65	311	317	−41.0° (0.999%)	$C_{15}H_{21}O_4NS$[c]	57.86	6.80	4.50	57.82	6.66	4.44
Glycine	A NaOH	EtOAc–petr. ether	77	88.5–89	175	174		$C_7H_{13}O_4N$	47.99	7.48	8.00	47.82	7.65	8.09
L-Isoleucine (hemihydrate)	A NaOH	Acetone–H_2O	9.6	49–57	240	242	+ 3.0 ± 0.5° (2.005%)	$C_{11}H_{22}O_{4.5}N$	55.10	9.22	5.82	55.39	9.29	5.95
L-Leucine (monohydrate)	B NaOH	EtOH–H_2O	59	67–72	249	252	−24° (2.002%)	$C_{11}H_{23}O_6N$	52.99	9.30	5.62	52.83	9.03	5.65
ε-Carbobenzoxy-L-lysine	B NaOH	An oil	62		380	393								
DL-Methionine	A Na_2CO_3	EtOAc–petr. ether	46	93.5–94.5	249	252		$C_{10}H_{19}O_4NS$[d]	48.17	7.68	5.62	48.29	7.98	5.56
L-Methionine	A Na_2CO_3	An oil	40		249	266								
DL-Phenylalanine	A NaOH	Ether–petr. ether, EtOAc–petr. ether	32	144.5–145	265	264		$C_{14}H_{19}O_4N$	63.38	7.22	5.28	63.16	7.30	5.14
L-Phenylalanine	A Na_2CO_3	EtOAc–petr. ether	73	79–80	265	266	− 0.8° (4.957%)	$C_{14}H_{19}O_4N$	63.38	7.22	5.28	63.62	7.28	5.25
L-Proline	A NaOH	Me Et ket.–petr. ether	55	136–137	215	215	−60.2° (2.011%)	$C_{10}H_{17}O_4N$	55.80	7.96	6.51	55.87	7.93	6.44
DL-Serine	B NaOH	An oil	29		205	215		$C_3H_{15}O_5N$	46.82	7.37	6.83	46.07	7.46	6.86
L-Tryptophan	A Na_2CO_3	EtOAc–petr. ether	36	136.5–140.5 d.	304	311	−18.2° (1.978%)	$C_{16}H_{20}O_4N_2$	63.16	6.63	9.21	63.07	6.52	9.24
L-Tyrosine	A $NaHCO_3$	EtOAc–petr. ether	29	136–138	281	288	+ 3.9 ± 0.5° (2.044%)	$C_{14}H_{19}O_5N$	59.77	6.81	4.98	60.10	7.06	4.95
L-Valine	A Na_2CO_3	Petr. ether	1.4[b]	77–79	217	217	− 5.8° (1.208%)	$C_{10}H_{19}O_4N$	55.32	8.73	6.45	55.58	9.13	6.34

[a] In most cases, crude yields were considerably higher than the pure yields reported here, and the melting points were not greatly lower. [b] A crude yield of 54%, with m.p. 73–77°, was obtained. Much difficulty was encountered in recrystallization. [c] Sulfur calcd. 10.30, found 10.34. [d] Sulfur calcd. 12.86, found 13.24.

cohol. Reaction of the carbonate with salts of amino acids occurs under mild conditions, and the resulting *t*-BOC-amino acids are isolated directly in a state of high purity

Table I shows that the *t*-BOC-amino acids in general are crystalline. It is likely that further work with those which are reported as oils will give crystalline products. The *t*-BOC-amino acids behave normally in peptide syntheses as shown by several examples.

Removal of the *t*-BOC group by hydrogen bromide or hydrogen chloride in acetic acid or in diethyl phosphite occurs more readily than the similar removal of the benzyloxycarbonyl group. Of special interest also is the resistance of the *t*-BOC group to catalytic hydrogenation and to sodium in liquid ammonia; this property should be of value in the synthesis of complex peptides where the use of a combination of protecting groups and their selective removal is desirable.

A new pyrophosphite reagent, diethyl ethylenepyrophosphite, was used in the synthesis of the *t*-BOC peptide esters. This reagent appears to be equivalent to the previously reported tetraethyl pyrophosphite[3] for formation of the peptide bond, and it has the advantage of being more readily prepared.

Experimental[14]

Reaction of *t*-Butyl N-Phenylcarbamate with Hydrogen Chloride.—*t*-Butyl N-phenylcarbamate, m.p. 135–136°, was prepared by the reaction of phenyl isocyanate with *t*-butyl alcohol.[6] It was treated then in 0.010-mole quantities with 0.01 mole quantities of hydrogen chloride in 7 ml. of diethyl phosphite.[15] Room temperature reactions for 1.5 and 4 hr. gave, respectively, 46 and 56% yields of aniline hydrochloride, which was precipitated by the addition of anhydrous ether; 52 and 42% recoveries of the carbamate were isolated from the filtrates. A similar reaction carried out by heating for 15 minutes on a steam-bath yielded 54% of aniline hydrochloride. A reaction of 0.010 mole of carbamate with 14 ml. of diethyl phosphite containing 0.02 mole of hydrogen chloride, conducted by 15 min. heating on a steam-bath, yielded 89% of aniline hydrochloride and a 5% recovery of the carbamate.

A qualitative reaction of *t*-butyl N-phenylcarbamate with concd. hydrochloric acid gave immediate gas evolution at room temperature. Hydrogen chloride in benzene also reacted with the carbamate on warming.

Reaction of Benzyl N-Phenylcarbamate with Hydrogen Chloride.—To a solution of 0.02 mole of hydrogen chloride in 7 ml. of diethyl phosphite was added 2.27 g. (0.010 mole) of benzyl N-phenylcarbamate.[8] After heating for 15 minutes on a steam-bath, the solution was cooled and diluted with

(14) Melting points were determined on a calibrated Fisher–Johns block. Analyses were done by L. Brancone and associates, and polarimetry by W. Fulmor.

(15) Solutions of hydrogen chloride in diethyl phosphite were made with cooling in ice water, then titrated for chloride content. These lost about 10% of titratable chloride per day when kept in a refrigerator.

ether. No precipitate formed. The ether was evaporated and the solution was diluted with 100 ml. of water, yielding 1.91 g. (84% recovery) of benzyl N-phenylcarbamate.

t-Butyloxycarbonylglycine by the Isocyanate Method.—Carbethoxymethyl isocyanate[16] (0.37 mole), *t*-butyl alcohol[16] (1.0 mole) and triethylamine[16] (0.37 mole) were combined and refluxed for 2.5 hours with exclusion of moisture, then allowed to stand overnight at room temperature. Excess volatile materials were removed by distillation under aspirator vacuum at steam-bath temperature. The crude, oily ethyl *t*-butyloxycarbonylglycinate residue was saponified by a two-hour reaction at room temperature with 100 ml. of dioxane and 100 ml. of 15% sodium hydroxide solution. After acidification with concentrated hydrochloric acid, the crude *t*-BOC-glycine was extracted out with three 100-ml. portions of ether and obtained as a solid, m.p. 80–85°, wt. 55 g. (85% yield) on evaporation of the ether. This was dissolved in 110 ml. of warm ethyl acetate, filtered from some high melting residue, treated with decolorizing carbon and refiltered, then diluted with 150 ml. of petroleum ether to cause crystallization. Recrystallization from 50 ml. methyl ethyl ketone yielded 24.7 g. (38%), m.p. 89–90°. Further quantities were recovered from the filtrates.

p-Nitrophenyl Chloroformate (by A. Pellicano).—Gaseous phosgene from a cylinder was bubbled through concd. sulfuric acid and condensed into a flask fitted with a Dry Ice–acetone condenser. When 223 ml. (about 3.1 moles) had been collected, 1400 ml. of benzene was added. Dry sodium *p*-nitrophenolate[17] (453 g., 2.8 moles) was then added in portions from a flask attached by a rubber tube. The reaction flask was cooled during the addition to avoid excessive refluxing of phosgene (the reaction temperature did not exceed 40°), then the mixture was allowed to reflux gently for an hour. Excess phosgene was distilled out while heating the flask by a water-bath at 50–60° for 30 minutes. Sodium chloride was removed by filtration, washed with benzene and the filtrates were concentrated under vacuum to 400 ml., then distilled. The product, b.p. 115–128° at 0.8 to 2.5 mm., solidified and was recrystallized from one liter of carbon tetrachloride; yield 68%, m.p. 81–82°. The literature[13] value is 81–82°.

t-Butyl *p*-Nitrophenylcarbonate.—*p*-Nitrophenyl chloroformate (prepared as described above) (0.51 mole, 103.5 g.) was added slowly in portions to a stirred solution of *t*-butyl alcohol[16] (0.51 mole, 38 g.) in 206 ml. pyridine at 0–5°. The reaction mixture was stirred at room temperature for 3 hr. and the precipitated pyridine hydrochloride was then removed by filtration; 25 ml. of water was added to the filtrate and the solution was extracted three times with 200 ml. of ether. The ether solution was washed three times each with 200-ml. portions of *N* hydrochloric acid, saturated sodium carbonate solution, and saturated sodium chloride solution. The ethereal extract was dried over sodium sulfate and evaporated to dryness. The residue was dissolved in 350 ml. of ethanol and the product precipitated by the addition of 400 ml. of water; yield 72%, m.p. 78.5–79.5°.

Anal. Calcd. for $C_{11}H_{13}O_5N$: C, 55.22; H, 5.48; N, 5.86. Found: C, 55.53; H, 5.71; N, 5.91.

t-Butyloxycarbonyl-L-phenylalanine (Method A).—A mixture of 1.65 g. (0.010 mole) of L-phenylalanine, 3.59 g. (0.015 mole) of *t*-butyl *p*-nitrophenylcarbonate, 2.65 g. (0.025 mole) of sodium carbonate, 15 ml. of *t*-butyl alcohol and 10 ml. of water was refluxed by steam-bath heat for 30 minutes. Two liquid layers persisted but all solids dissolved during this period. The condenser was removed and the mixture was concentrated by an air stream during 10 minutes heating to remove *t*-butyl alcohol. Sodium *p*-nitrophenolate dihydrate crystallized and was collected after cooling and washed with 7 ml. of water in 3 portions; the yield of the air-dried salt was 88%. The filtrate was adjusted to *p*H 5 to 6 by dilute hydrochloric acid and extracted with two 20-ml. portions of ether to remove any remaining *t*-butyl *p*-nitrophenylcarbonate and *p*-nitrophenol. The aqueous portion was then adjusted to *p*H about 1 and the *t*-BOC-L-phenylalanine was extracted into three 10-ml. portions of anhydrous ether. After evaporation of the ether, the solid residue was recrystallized from 60 ml. of petroleum ether (30–60°) plus 4 ml. of ethyl acetate; yield 1.94 g. (73%), m.p. 79–80°. Yields were almost as good when a 25% excess of *t*-butyl *p*-nitrophenyl carbonate was used instead of 50%, and were somewhat lower with no excess. Sodium hydroxide or sodium bicarbonate was satisfactory in place of sodium carbonate; 15 minutes appeared to be a minimum reaction time under these conditions.

t-Butyloxycarbonyl-L-leucine (Method B).—A mixture of 6.55 g. (0.050 mole) of L-leucine, 14.95 g. (0.063 mole) of *t*-butyl *p*-nitrophenyl carbonate, 57 ml. of 2 *N* sodium hydroxide and 100 ml. of *t*-butyl alcohol was stirred at room temperature. Most of the solids had dissolved in an hour, and crystalline sodium nitrophenolate dihydrate formed soon after. After 29 hours, the mixture was filtered and *t*-butyl alcohol was removed from the filtrate by evaporation in an air stream while warming the solution to about 35°, giving a further precipitate. This was collected and washed with 10 ml. of 5% sodium bicarbonate, then a little water. The combined solids were dried and extracted with ether to give an 11% recovery of *t*-butyl *p*-nitrophenylcarbonate.

The aqueous filtrate was neutralized to *p*H 7, and a small amount of leucine precipitated. Extraction of the filtrate from this with 90 ml. of a-ether in two portions gave 0.7 g. of a sticky solid (discarded). Then acidification of the aqueous solution to *p*H about 1 gave an immediate precipitate of *t*-BOC-L-leucine hydrate which weighed 8.16 g. (65% yield) after air drying. It was recrystallized by the addition of 100 ml. of water to its solution in 50 ml. of ethanol; yield 7.31 g. (59%), m.p. 67–72° with softening around the edges of the sample at about 61°. This hydrated compound is unusual in being hydrophobic. Drying in vacuum gave the anhydrous compound as an oil which could not be induced to crystallize.

A similar reaction run for 19 hours gave a 60% crude yield, 53% pure.

Diethyl Ethylenepyrophosphite.—A solution of 238 g. (1.72 moles) of diethyl phosphite[18] and 175 g. (1.72 moles) of triethylamine in 345 ml. of reagent grade benzene was cooled to 0° and a solution of 218 g. (1.72 moles) of ethylene chlorophosphite[19] in 345 ml. of benzene was added over a period of one hour. During this time and for 1.75 hours thereafter, the temperature was kept at 0 ± 5°. The mixture was filtered to remove triethylammonium chloride and the filtrate was distilled through a 10-inch Vigreux head, giving a main fraction of 314 g., b.p. 64–75° at 0.35 mm. Redistillation gave 216 g. (55% yield), b.p. 65–73° (mostly 68–69°) at 0.4 mm., $n^{26}D$ 1.4521. Caution: overheating of residual pot material can cause exothermic polymerization. Another preparation gave b.p. 95–97° at 5 mm., $n^{26}D$ 1.4529. Arbuzov[20] prepared the compound from the sodium salt of diethyl phosphite, and reported b.p. 84–85° at 2 mm., $n^{20}D$ 1.4557.

Ethyl DL-Phenylalaninate Hydrobromide.—DL-Phenylalanine (49.5 g.) in 300 ml. of anhydrous ethanol was treated with hydrogen bromide, causing solution within 10 minutes with heat evolution. After an hour, hydrogen bromide addition was stopped and the solution was refluxed for two hours. Ethanol was removed in an hour under vacuum, and the resulting solid was recrystallized by solution in 300 ml. of hot acetone, filtering, diluting with 500 ml. of diisopropyl ether and refrigerating: yield 65 g. (79%), m.p. 130–132°. A sample again recrystallized with 94% recovery gave m.p. 131–132°.

Anal. Calcd. for $C_{11}H_{16}BrNO_2$: C, 48.2; H, 5.88; Br, 29.2; N, 5.11. Found: C, 48.3; H, 6.01; Br, 28.9; N, 5.39.

Ethyl *t*-Butyloxycarbonylglycyl-DL-phenylalaninate.—To a mixture of 2.10 g. (0.012 mole) of *t*-butyloxycarbonylglycine and 2.74 g. (0.010 mole) of ethyl DL-phenylalaninate hydrobromide was added 3 ml. of diethyl phosphite,[18] 4 ml. of trimethyl phosphite[18] as hydrogen bromide acceptor[21] and 2.3 ml. (0.012 mole) of diethyl ethylenepyrophosphite. The flask was fitted with a condenser and a magnetic stirring bar was used. It was then immersed in a bath and the temperature was maintained at 114–116° for five minutes

(16) Purchased from Eastman Kodak Co.

(17) The dihydrate purchased from Eastman Kodak Co. was dehydrated by heating in a forced draft oven at 120°; the material changed from yellow to red upon loss of water.

(18) Purchased from Virginia–Carolina Chemical Co.

(19) H. J. Lucas, F. W. Mitchell and C. N. Scully, THIS JOURNAL, **72**, 5491 (1950).

(20) B. A. Arbuzov, K. V. Nikonorov, O. N. Sedorova, G. M. Vinokurova and Z. G. Shishova, *Doklady Akad. Nauk (S.S.S.R.)*, **91**, 817 (1953).

(21) R. W. Young, K. H. Wood, R. J. Joyce and G. W. Anderson, THIS JOURNAL, **78**, 2126 (1956).

with stirring. The resulting solution was cooled and diluted with 50 ml. of water to precipitate the product as an oil which crystallized on chilling. It was collected, washed with water, a 5% solution of sodium bicarbonate, and water; yield 3.05 g. (87%), m.p. 99–101°. Recrystallization from ethyl acetate (15 ml.) and petroleum ether (100 ml.) gave 2.56 g. (73%), m.p. 102–103°. The same crude yield was obtained when a similar reaction with only a 10% excess of *t*-BOC-glycine was used.

Anal. Calcd. for $C_{18}H_{26}N_2O_5$: C, 61.7; H, 7.43; N, 8.00. Found: C, 61.8; H, 7.71; N, 8.17.

Ethyl *t*-Butyloxycarbonyl-L-phenylalanylglycinate.—A mixture of 2.65 g. of *t*-butyloxycarbonyl-L-phenylalanine, 1.40 g. of ethyl glycinate hydrochloride, 1.4 ml. (1.0 g.) of triethylamine, 7 ml. of diethyl phosphite and 2.50 g. of diethyl ethylenepyrophosphite was heated for 15 minutes on a steam-bath, cooled and the product precipitated by the addition of 25 ml. of water. After washing with water, 10 ml. of 5% sodium bicarbonate solution, then water, the crystalline solid was dried in a steam cabinet, yielding 2.85 g. (81%), m.p. 86–87°. Two recrystallizations by solution in a few ml. of ethyl acetate and addition of petroleum ether gave 1.24 g., m.p. 89.5–90°, having $[\alpha]^{25}D$ −4.3° (*c* 2, ethanol).

Anal. Calcd. for $C_{18}H_{26}N_2O_5$: C, 61.7; H, 7.48; N, 8.00. Found: C, 61.9; H, 7.70; N, 8.32.

Reaction of Ethyl *t*-Butyloxycarbonyl-L-phenylalanylglycinate with Hydrogen Bromide (A).—A saturated solution of hydrogen bromide in 2.5 ml. of diethyl phosphite was made by slowly bubbling in the gas while chilling in an ice–methanol-bath, giving approximately 3.5 ml. of 9 *M* solution.[22] This was then added to a solution of 3.50 g. of the peptide derivative in 7 ml. of diethyl phosphite. The mixture became warm, and bubbling which occurred was over in approximately a minute. The solution was diluted to cloudiness with anhydrous ether (about 75 ml.) and ethyl L-phenylalanylglycinate hydrobromide soon crystallized; wt. 2.76 g. (84% yield), m.p. 133.5–134°. A similar sample prepared on a smaller scale in 90% yield had m.p. 134–135°, $[\alpha]^{25}D$ +41.8° (*c* 1, water); a mixed m.p. with an authentic sample[3] was not depressed.

(B).—Crude ethyl *t*-butyloxycarbonyl-L-phenylalanylglycinate was synthesized by the pyrophosphite "anhydride" procedure.[3] A solution of 3.18 g. (0.012 mole) of *t*-butyloxycarbonyl-L-phenylalanine in 2 ml. of diethyl phosphite and 2.75 g. (0.012 mole) of diethyl ethylenepyrophosphite was made by warming on a steam-bath for a minute or so. This was added to a warmed mixture of 1.84 g. (0.010 mole) of ethyl glycinate and 4 ml. of trimethyl phosphite. The reaction was completed by heating for 15 minutes on a steam-bath, and the product was precipitated from the cooled solution by the addition of 50 ml. of water. It was collected, washed with 10 ml. of water, 10 ml. of 5% sodium bicarbonate solution and two 5-ml. portions of water. The dry weight was 3.32 g. (95% yield), m.p. 80–85°. Half of this was added to a saturated solution of hydrogen bromide in 8 ml. of diethyl phosphite (made by saturation while chilling) and the mixture was warmed on a steam-bath for a minute or so to complete the reaction. The ethyl L-phenylalanylglycinate hydrobromide was precipitated as a solid by the addition of anhydrous ether; m.p. 105–112° dec. It was dissolved in 3 ml. of warm ethanol and precipitated by anhydrous ether, giving 1.08 g. (65% over-all yield), m.p. 133–134°.

(C).—Hydrogen bromide was bubbled into 5 ml. of glacial acetic acid while cooling in an ice–water-bath. Then 0.92 g. of ethyl *t*-butyloxycarbonyl-L-phenylalaninate was added at room temperature, causing immediate effervescence which was over in a few seconds. About 100 ml. of anhydrous ether was added and the product crystallized in 86% yield, m.p. 131–133°.

Ethyl *t*-Butyloxycarbonylglycyl-L-phenylalanylglycinate.—A mixture of 2.76 g. of ethyl L-phenylalanylglycinate hydrobromide, 1.75 g. of *t*-butyloxycarbonylglycine, 1.7 ml. of trimethyl phosphite, 1.7 ml. of diethyl phosphite and 2.1 ml. (0.011 mole) of diethyl ethylenepyrophosphite was heated for 15 minutes on a steam-bath with protection from moisture. The resulting solution was poured into 50 ml. of water and the resulting oil was separated and washed with water, 10 ml. of 5% sodium bicarbonate solution and 20 ml. of water. The crystalline product which resulted weighed 2.25 g. and had m.p. 94–97°. Recrystallization from 10 ml. of ethanol plus 25 ml. of water yielded 1.96 g. (57%), m.p. 98–99°. Recrystallization from ethyl acetate–petroleum ether did not raise the melting point; $[\alpha]^{25}D$ −10.9° (*c* 2, methanol).

Anal. Calcd. for $C_{20}H_{29}N_3O_6$: C, 59.0; H, 71.7; N, 10.3. Found: C, 58.6; H, 7.08; N, 10.6.

***t*-Butyloxycarbonylglycyl-DL-phenylalanine.**—A solution of 7 g. of ethyl *t*-butyloxycarbonylglycyl-DL-phenylalaninate in 30 ml. of dioxane was added to a solution of 0.88 g. of sodium hydroxide in 25 ml. of water. After 15 minutes at room temperature the solution was acidified with concentrated hydrochloric acid and the product was extracted into 100 ml. of ether (3 portions). An oil obtained by evaporation of the ether solidified after washing with petroleum ether; wt. 6.22 g. (97% yield), m.p. 131.5–132.5°. Recrystallization from 50 ml. of ethyl acetate by the addition of 50 ml. of petroleum ether gave 5.73 g. having the same melting point.

Anal. Calcd. for $C_{16}H_{22}N_2O_6$: C, 59.6; H, 6.88. Found: C, 59.46; H, 7.48.

The same compound was obtained by the reaction of glycyl-DL-phenylalanine with *t*-butyl *p*-nitrophenyl carbonate (method A).

Attempted Reactions of *t*-Butyloxycarbonylglycyl-DL-phenylalanine with Sodium in Liquid Ammonia.—A solution of 1.5 g. of the dipeptide derivative in 100 ml. of liquid ammonia was treated with small portions of sodium until the blue color persisted for over 30 seconds after the last addition. The ammonia was allowed to evaporate and the residue was dissolved in 10 ml. of water. After acidification to about *p*H 1, the solution was extracted with 60 ml. of ether in three portions. Evaporation of the ether left 1.34 g. (89% recovery) of the *t*-BOC peptide, m.p. 130–132° dec.

Attempted Hydrogenation of Ethyl *t*-Butyloxycarbonylglycyl-DL-phenylalaninate.—Three and a half grams of the dipeptide derivative was added to 50 ml. of ethanol (not all dissolved) and about 1 ml. of wet palladium catalyst and 1 ml. of glacial acetic acid were added. Hydrogen was bubbled through for two hours, and no carbon dioxide could be detected in the effluent gas at any time. The mixture was filtered and the unreacted peptide derivative was precipitated by the addition of water; weight 2.95 g. (84% recovery), m.p. 99–102°.

A similar reaction with ethyl carbobenzoxyglycyl-DL-phenylalaninate gave immediate carbon dioxide evolution.

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(22) Hydrogen bromide reacts with diethyl phosphite at an appreciable rate above room temperature; a solution prepared without chilling was found to have very little hydrogen bromide (by titration after adding water).