group occupies the same position of the peptide chain in both mercaptalbumin and non-mercaptalbumin.

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t-Butyl Esters of Amino Acids and Peptides and their Use in Peptide Synthesis[1]

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The synthesis of *t*-butyl esters of amino acids and peptides and their use in peptide synthesis is described. The most convenient method was the acid-catalyzed reaction of isobutylene with benzyloxycarbonylamino acids or peptides followed by catalytic hydrogenation to produce the basic esters. Another general method entailed the use of silver salts and *t*-butyl iodide. *t*-Butyl esters are particularly useful in peptide synthesis in that the ester group may be removed by acid catalysis and thus side reactions encountered in alkaline hydrolysis may be avoided. Other advantages arise from the stability of amino acid or peptide esters as free bases, particularly in allowing their storage and use as such. Comparative stabilities to ethyl esters are reported in several examples. The synthesis of several *t*-butyl peptides is reported as well as selective removal of this ester group or of amine-protecting groups when both are present.

In recent years naturally occurring peptides with high biological activities in such diverse fields as antibiotics, bacterial growth factors, hormones, smooth muscle stimulants and pain-producing substances have been isolated. Synthesis of such peptides has not kept up with structure determination largely because present methods are time-consuming and frequently give poor yields.[2] As part of an investigation of synthetic methods, the work reported here was directed toward improvements of carboxyl-protecting groups.

It has been found that *t*-butyl esters of amino acids and peptides have advantages over the customarily used methyl and ethyl esters. Specifically, they are much more stable as the free bases to self-condensation reactions (Table IV) and thus may usually be stored and used as such. This avoids the customary neutralization of hydrohalide salts with bases such as triethylamine during a peptide synthesis, a complicating procedure which adds to the danger of racemization in sensitive cases as well.[3,4] Perhaps more important, the many side reactions of saponification of peptide esters[2] can be avoided since the *t*-butyl group is readily removed by acid catalysis under mild conditions. In comparison to benzyl esters, *t*-butyl esters are much more readily removed by acid catalysis and are not affected by hydrogenation in the presence of palladium or platinum.

Two general procedures for the synthesis of amino acid and peptide *t*-butyl esters have been investigated. In both an amine-protecting group which was subsequently removed was used. In spite of the extra steps necessary it seems likely that the utility of *t*-butyl esters will make this worth while.

The first method entailed the reaction of silver salts of acylamino acids or acylpeptides with *t*-butyl iodide (example 1 in Experimental and Table I). Although this is straightforward, the second method is simpler, gives better yields and is preferable for large-scale reactions. In the latter, acylamino acids or acylpeptides were treated with a large excess of isobutylene in the presence of sulfuric acid or *p*-toluenesulfonic acid as catalyst. The products were isolated by treatment with aqueous alkali (example 2 and Table I). The preferred amine-protecting group in this investigation was benzyloxycarbonyl (carbobenzoxy) but others were used (see experimental).

The benzyloxycarbonyl group was removed by hydrogenation and phosphite salts were prepared for purification and characterization. Since phosphorous acid is weak, danger of cleavage of the *t*-butyl group was avoided (example 3 and Table II). In some cases, phosphite salts were not isolated, but were converted to the free bases (example 4 and Table III).

Several peptides were synthesized from amino acid *t*-butyl esters by the tetraethyl pyrophosphite,[3] dicyclohexylcarbodiimide[5] and *p*-nitrophenyl ester[6] procedures.

Since commonly used amine-protecting groups have varying degrees of sensitivity to removal by acids, selective or simultaneous removal with *t*-butyl esters is possible. Thus refluxing with *p*-toluenesulfonic acid in benzene was used to remove selectively *t*-butyl esters in the presence of benzyloxycarbonyl or trifluoroacetyl groups (examples 6 and 8B). A stronger acid, hydrogen bromide in glacial acetic acid, does not affect phthaloyl groups (example 11) or trifluoroacetyl groups at 10° (example 8A), but it will simultaneously remove benzyloxycarbonyl or *t*-butyloxycarbonyl groups along with *t*-butyl esters (example 15).

The trifluoroacetyl group can be selectively removed from trifluoroacetylpeptide *t*-butyl esters

(1) Presented in part at the 133rd Meeting of the American Chemical Society at San Francisco, Calif., April, 1958. During preparation of this manuscript, a brief note on amino acid *t*-butyl esters appeared (R. W. Roeske, *Chemistry & Industry*, Sept. 5, 1959).

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TABLE I

BENZYLOXYCARBONYLAMINO ACID *t*-BUTYL ESTERS, Z-R-O-*t*-Bu[a]

R	Method	Yield, %	$[\alpha]^{25}D$ (*c*, EtOH)	Physical constants	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
DL-ala[b]	*i*-Bu	46.8	.....	$n^{25}D$ 1.4877, d^{25}_{25} 1.0710	..	..	..	..	..	..
gly	Ag	36	.....	B. 162–165° (0.4 mm.), $n^{25}D$ 1.4960	63.4	7.22	5.28	63.7	7.38	5.33
gly	*i*-Bu	69	.....	$n^{29}D$ 1.4976	..	..	..	..	..	..
L-glu	*i*-Bu	95	.....	$n^{25}D$ 1.4867, d^{25}_{25} 1.1032	..	..	..	..	..	..
L-glu-γ-NH_2	*i*-Bu	64	−10.9° (5)	M. 94–95°	60.7	7.19	8.33	60.6	7.12	8.23
L-leu	*i*-Bu	94	.....	$n^{25}D$ 1.4853, d^{20}_{4} 1.0428	..	..	..	..	..	..
L-leu	Ag	9	.....	$n^{26}D$ 1.4853, d^{25}_{25} 1.0532	..	..	..	..	..	..
L-ileu	*i*-Bu	86	−8.8 (3.1)	$n^{20}D$ 1.4865, d^{20}_{4} 1.0473	67.3	8.47	4.36	67.1	8.71	4.42
L-met[c]	*i*-Bu	82	−27 (5.7)	$n^{20}D$ 1.5190, d^{20}_{4} 1.1226	60.2	7.42	4.13	59.6	7.36	4.27
L-phe	Ag	30	−4.4 (2)	M. 81–82° ($MeOH-H_2O$)	71.0	7.09	3.94	71.0	7.21	3.89
L-phe	*i*-Bu	50[d]	−4.6 (2)	M. 80.5–81.5° (diisopropyl ether)	..	..	..	..	..	..
DL-phe[b]	*i*-Bu	61	.....	$n^{28}D$ 1.5196, d^{28}_{28} 1.0914	..	..	..	..	..	..
D-phe	*i*-Bu	90	+4.5 (2)	M. 80–81°	71.0	7.09	3.94	70.3	7.24	4.01
L-pro	*i*-Bu	95	−52.5 (2.2)	M. 44–45° (ether–petr. ether)	66.9	7.59	4.59	66.7	7.89	4.46
L-tyr[b]	*i*-Bu	56	.....	$n^{26.5}D$ 1.5222, $d^{26.5}_{26.5}$ 1.1212	..	..	..	..	..	..
DL-val[b]	*i*-Bu	59	.....	$n^{27}D$ 1.4806, d^{27}_{27} 1.4806	..	..	..	..	..	..
L-val	*i*-Bu	97	.....	$n^{20}D$ 1.4887, d^{20}_{4} 1.0580	66.4	8.20	4.56	66.3	7.99	4.76

[a] See examples 1 and 2 for procedures used. [b] Reaction carried out in methyl isopropyl ketone. [c] Calcd.: S, 9.44. Found: 9.78. [d] Crude yield 71%, m.p. 78–80°; this is suitable for the reduction step.

TABLE II[a]

PHOSPHITE SALTS OF AMINO ACID *t*-BUTYL ESTERS, H_3PO_3-H-R-O-*t*-Bu[a]

R	Yield from Z. deriv.,[b] %	M.p., °C.	Recrystn. solvent	$[\alpha]^{25}D$ (*c*, H_2O)	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
NH_2 | L-Glu	74	123–124	MeOH–$(i\text{-}Pr)_2O$	+15.3° (4.59)	38.0	7.44	9.86	38.0	7.52	9.82
gly	82	155–157 d.	MeOH–$(i\text{-}Pr)_2O$		33.8	7.56	6.57	34.1	7.89	6.71
L-leu	70	163–164 d.	EtOH–$(i\text{-}Pr)_2O$	+5.0 (5)	44.6	8.92	5.20	44.6	9.15	5.20
L-phe	77	156–158 d.	MeOH–$(i\text{-}Pr)_2O$	+3.1 (4.58)	51.4	7.31	4.62	50.8	7.38	4.63
DL-phe	72	168–169 d.	*i*-PrOH		51.4	7.31	4.62	51.4	7.29	4.62
L-tyr	35	167.5 d.[c]	MeOH–$(i\text{-}Pr)_2O$	+4.4 (5.01)	48.9	6.94	4.39	48.9	6.71	4.35
DL-val	55	138–139 d.	MeCOEt		42.4	8.69	5.49	42.5	8.81	5.56

[a] See example 3 for procedure used. [b] Z = benzyloxycarbonyl (carbobenzoxy). [c] The pure compound decomposed sharply at 167.5° without melting, presumably losing isobutylene; the remaining product then melted 185–188°.

TABLE III

AMINO ACID *tert*-BUTYL ESTERS, H-R-O-*t*-Bu[a]

R	Yield from Z. deriv. or phosphite salts[b]	B.p. (mm.) or m.p., °C.	$[\alpha]^{25}D$ (*c*, EtOH)	$n^{26}D$	d^{20}_{4}	*MRD* Calcd.	*MRD* Found	Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
L-glu[d]	60 (Z)	110 (0.05)	+16.6° (5.4)	1.4378	0.992	69.1	69.1	60.2	9.72	5.40	60.0	9.64	5.69
gly	83 (P)	30 (2)	.....	1.4239	0.960	35.1	34.8	54.9	10.0	10.7	54.8	10.0	10.3
L-ileu	82 (Z)	52 (0.45)	+26.7 (100%)	1.4309	0.909	53.6	53.3	64.1	11.3	7.48	64.6	11.6	7.43
L-leu	85 (Z)	45 (0.15)	+21.6 (2.5)	1.4254	0.899	53.6	53.3	64.1	11.3	7.48	63.9	11.8	7.91
D-phe	86 (Z)	115 (0.75)[c]	−24.8 (100%)	1.5006	1.016	63.8	64.0	70.6	8.65	6.33	70.3	8.94	6.31
DL-phe	77 (P)	96 (0.35)	.....	1.4536	1.014	63.8	64.2	..	...	...	...	...	...
L-phe	86 (Z)	107 (0.25)[c]	+24.6 (100%)	1.4970	1.015	63.8	63.8	70.6	8.65	6.33	70.3	8.61	6.63
L-pro	77 (Z)	57 (1.5)	−41.5 (1.8)	1.4421	0.971	46.8	46.7	63.1	10.0	8.18	62.4	10.2	8.16
L-tyr	50 (P)	142–143	.....	.....	.....	..	..	65.8	8.07	5.90	65.4	8.14	5.90
L-val	88 (Z)	63 (1.25)	+25.5 (100%)	1.4265	0.912	49.0	48.7	62.4	11.1	8.09	62.5	11.2	8.17

[a] See example 4. [b] Z is benzoyloxycarbonyl, P is phosphite salt. [c] M.p. 6°. [d] Diester.

by alkali treatment; this combination of protecting groups could be very useful (example 9B).

Selective removal of a *t*-butyloxycarbonyl group in the presence of a *t*-butyl ester should be possible with trifluoroacetic acid. The removal of phthaloyl groups in the presence of ethyl or methyl esters by hydrazine is not practical because the esters also react; the resistance of *t*-butyl esters to aminolysis raises the possibility that phthaloyl groups can be removed in their presence. These possibilities are under investigation.

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Experimental[7]

Example 1. Preparation of *t*-Butyl Benzyloxycarbonyl-L-phenylalaninate by the Silver Salt Method (see Table I).—Benzyloxycarbonyl-L-phenylalanine[8] (m.p. 75–76°, 54.5 g.) was dissolved in an excess of concentrated ammonium hydroxide, and the solution was concentrated under vacuum to near dryness. Isopropyl alcohol was added and the concentrating process repeated. Water was added (200 ml.) and the resulting solution was combined with a solution of silver nitrate (30.9 g. in 200 ml. of water). The resulting precipitate of silver benzyloxycarbonyl-L-phenylalaninate was collected and dried in a steam oven; wt. 74 g. (88% yield), m.p. 183–188° dec. This was suspended in 500 ml. of anhydrous ethyl ether, and 35 g. of *t*-butyliodide[9] was slowly added. The precipitate of silver iodide was removed and washed with anhydrous ether. Evaporation of the combined ether portions in an open dish left *t*-butyl benzyloxycarbonyl-L-phenylalaninate (20 g., 33% yield from Ag salt) as a crystalline solid. This was recrystallized by making a solution in 100 ml. of warm methanol and adding this to 100 ml. of water containing 2 g. of sodium bisulfite; yield 19 g., m.p. 79–80°. A 1-g. sample was recrystallized from 3 ml. of diisopropyl ether to yield 0.90 g., m.p. 81–82°, $[\alpha]^{25}D$ −4.4° (*c* 2, ethanol).

2. Preparation of *t*-Butyl Benzyloxycarbonyl-L-prolinate by the Isobutylene Method.—Three ml. of concd. sulfuric acid was added to a solution of 74.3 g. (0.30 mole) of benzyloxycarbonyl-L-proline[10] in 600 ml. of methylene chloride. The solution was saturated with isobutylene, causing a volume increase of 300 ml. After 65 hours at room temperature, the solution was added to 500 ml. of water containing sodium carbonate sufficient to neutralize all acids. The methylene chloride layer was separated, washed with water, then concentrated under vacuum at 60° to an oil which crystallized; weight 86.5 g. (95% of theory), m.p. 44–45°. A sample recrystallized from petroleum ether–ethyl ether by chilling in a Dry Ice–acetone mixture had the same m.p.

3. *t*-Butyl L-Phenylalaninate Phosphite from the Benzyloxycarbonyl Derivative (see Table II).—Hydrogenation of 10.7 g. of Z·phe·O-*t*-Bu in100 ml. of ethanol with 1 g. of 10% palladium-on-charcoal catalyst was complete in 90 min. at room temperature and atmospheric pressure as measured by the disappearance of carbon dioxide in the exit gases. The mixture was filtered and 2.42 g. of crystalline phosphorus acid[11] was added, causing immediate crystallization of *t*-butyl L-phenylalaninate phosphite: wt. 7.0 g. (77% yield), m.p. 156–158°, $[\alpha]^{25}D$ + 3.1° (*c* 4.58, water). A sample recrystallized from methanol–diisopropyl ether had the same m.p.

4. *t*-Butyl L-Prolinate from the Benzyloxycarbonyl Derivative (see Table III).—Hydrogenation of 30.5 g. (0.10 mole) of Z·pro·O-*t*-Bu in 250 ml. of absolute ethanol in the presence of 3 g. of 10% Pd–C was accomplished in 3 hours. After removal of the catalyst, the resulting solution was concentrated to 75 ml. under vacuum and 8.2 g. (0.10 mole) of phosphorus acid in 200 ml. of ether was added. The phosphite salt precipitated as an oil so 250 ml. of water was added. The ether layer was separated and concentrated, giving 1.6 g. (5%) of unreacted Z·pro·O-*t*-Bu. The aqueous layer was made alkaline with 7 g. of NaOH and then extracted with two 100-ml. portions of ether. The combined ether solutions were dried over sodium sulfate, then concentrated under vacuum to yield 13.2 g. (77%) of an oil, b.p. approx. 57° at 1.5 mm.

5. *t*-Butyl Benzyloxycarbonylglycyl-L-phenylalaninate. A. By the Isobutylene Reaction.—Z·gly-phe·OH(L)[12] (18.3 g., $[\alpha]^{25}D$ + 38.5° (*c* 5, EtOH)) was suspended in 500 ml. of methylene chloride and 1 ml. of concd. sulfuric acid was added. Isobutylene was passed in for 2 hours with stirring, and the resulting solution was stored at room temperature for 66 hours. The *t*-butyl ester was then isolated by stirring the solution with 200 ml. of 5% potassium hydroxide solution, separating the organic layer, washing it with 100 ml. of water, drying over sodium sulfate, filtering through Celite and removing the solvent under a vacuum. A 19-g. portion of the resulting 22 g. of thick oil was dissolved in 20 ml. of diisopropyl ether, filtered through Celite and diluted with 100 ml. of *n*-heptane. The product, which rapidly crystallized, was separated after chilling an hour in ice-water and washed with diisopropyl ether; wt. 14 g. (75% yield), m.p. 61–63°. A sample recrystallized from methyl ethyl ketone by adding heptane had m.p. 62–63°, $[\alpha]^{25}D$ + 10.2° (*c* 2, EtOH). *Anal.* Calcd. for $C_{23}H_{28}N_2O_5$: C, 67.0; H, 6.84; N, 6.79. Found: C, 66.6; H, 7.13; N, 6.62.

B. By Peptide Synthesis Using Tetraethyl Pyrophosphite.—Z·gly·OH[13] (10.45 g.), H·phe·O-*t*-Bu (L) (11.05 g.) and 15 ml. of tetraethyl pyrophosphite were added to 35 ml. of dimethoxyethane (distilled from calcium hydride). The solution was refluxed for 30 min., then added to 250 ml. of of ice-water. The oil which formed crystallized on refrigerating overnight; yield 20.5 g. (99.5%), m.p. 60.5–62°. A 1-g. sample was recrystallized from 2 ml. of diisopropyl ether plus 10 ml. of *n*-heptane; yield 0.85 g., m.p. 61.5–63.5°.

6. Benzyloxycarbonylglycyl-L-phenylalanine from the *t*-Butyl Ester.—A 10.0-g. sample of the ester from 5A was refluxed in solution with 1 g. of *p*-toluenesulfonic acid in 75 ml. of benzene for an hour. The cooled solution was extracted with 300 ml. of 5% sodium bicarbonate solution. After filtration to remove a small amount of gum, acidification of the extracts gave a crystalline precipitate Z·gly-phe·OH(L). Recrystallization from 80.0 ml. of water yielded 6.29 g. (71%), m.p. 127–128°, $[\alpha]^{25}D$ + 36.6 ± 0.5° (*c* 5, ethanol).

7. *t*-Butyl Trifluoroacetylglycyl-L-phenylalaninate.—Trifluoroacetylglycine (0.010 mole), *t*-butyl L-phenylalaninate (0.010 mole) and tetraethyl pyrophosphite (0.011 mole) were added to 7 ml. of dry dimethoxyethane. The solution was refluxed for 30 minutes, cooled and diluted with 50 ml. of ice-water. The crystalline product which precipitated was collected, washed with water and dried; yield 3.60 g. (96%), m.p. 113–114°. Recrystallization was accomplished by dissolving in 15 ml. of isopropyl alcohol, filtering, and adding 50 ml. of water in portions; yield 3.10 g. (83%), m.p. 119–120°, $[\alpha]^{25}D$ + 7.2° (*c* 5, ethanol). *Anal.* Calcd. for $C_{17}H_{21}F_3N_2O_4$: C, 54.6; H, 5.66; N, 7.49; F, 15.2. Found: C, 54.3; H, 6.20; N, 7.80; F, 15.7.

8. Trifluoroacetylglycyl-L-phenylalanine from the *t*-Butyl Ester. A. Using Hydrogen Bromide.—Five ml. of glacial acetic acid was saturated with HBr at 10°. The ester (1.02 g.) was added; this promptly dissolved. After 2 minutes the solution was added to 25 ml. of ice-water, giving a slightly turbid solution. This was filtered after 10 minutes and concentrated under vacuum to 15 ml. The dipeptide acid rapidly crystallized at this point. It was collected after chilling an hour, and a further portion was obtained by concentration of the filtrate to 10 ml.; yield 0.60 g. (71%), m.p. 184–187°.

B. Using *p*-Toluenesulfonic Acid.—A solution of 1.12 g. of the *t*-butyl ester and 0.10 g. of *p*-toluenesulfonic acid in 5 ml. of benzene was refluxed for 30 minutes. The product began crystallizing during the reaction; it was collected after cooling and washed with 3 ml. of benzene; yield 0.72 g. (76%), m.p. 184–185°. A 450-mg. portion was recrystallized by dissolving in 2,2-dimethyldioxolane and adding 3 ml. of *n* heptane; yield 400 mg., m.p. 186.5–187°, $[\alpha]^{25}D$ + 41.2 ± 0.5° (*c* 4.9, ethanol). *Anal.* Calcd. for $C_{13}H_{13}F_3N_2O_4$: C, 49.1; H, 4.11; N, 8.80; F, 17.9. Found: C, 49.2; H, 4.41; N, 9.20; F, 16.8.

9. *t*-Butyl Glycyl-L-phenylalaninate. A. By Hydrogenation of the Benzyloxycarbonyl Derivative.—A portion (8.2 g.) of the product from 5A was hydrogenated in 100 ml. of ethanol with 2 g. of 10% Pd–C catalyst for an hour. After filtering, the resultant solution was treated with a solution of 1.64 g. of phosphorus acid in 50 ml. of ether. No precipitate occurred, so the solution was concentrated to 20-ml. volume. Addition of 300 ml. of ethyl ether and 300 ml. of diisopropyl ether precipitated a crude phosphite salt. This was added to 25 ml. of water containing an excess of sodium hydroxide, and the free base was extracted into 50 ml. of ether. After drying over sodium sulfate, the solvent was removed under vacuum, leaving an oil which slowly crystallized on refrigeration; weight 3.8 g. (66% yield as the hemihydrate), m.p. 34.5–35.5°. *Anal.* (after 18

(7) Melting points were taken on a Fisher–Johns block and are uncorrected.

(8) M. Goodman and K. C. Steuben, *J. Org. Chem.*, **24**, 112 (1959).

(9) Purified by washing with aqueous sodium bisulfite solution and drying over calcium chloride.

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months at −20°) Calcd. for $C_{15}H_{22}N_2O_2 \cdot 1/2H_2O$: C, 62.7; H, 8.07; N, 9.75. Found: C, 63.5; H, 8.23; N, 10.4. The m.p. was unchanged, $[\alpha]^{25}D$ + 17.3° (*c* 4, EtOH) and neutral equivalent 292 (theory 287). Solution in anhydrous ether–petroleum ether (1:1) gave a slight haze, indicating possibly a small amount of the diketopiperazine. A better analysis was obtained for the freshly prepared material in **B** below.

B. By Alkaline Hydrolysis of the Trifluoroacetyl Derivative.—TFA-gly-phe-O-*t*-Bu(L) (compd. 7, 3.16 g., 85 millimoles) was stirred with 15 ml. of *N* sodium hydroxide solution for 5 minutes. The oil which separated was extracted into 40 ml. of ether (two portions). The ether layer was separated and shaken with 25 ml. of 0.6 *N* hydrochloric acid. The product was then liberated from the aqueous phase by the addition of solid KOH and extracted into 20 ml. of ether (two portions). Removal of the ether under vacuum followed by drying by evacuation to 20 μ pressure for 7 hours left 1.56 g. (65% yield) of an oil, $n^{26}D$ 1.5094, which crystallized on refrigeration; m.p. 35–36°, $[\alpha]^{25}D$ +17.7° (*c* 4, ethanol). *Anal.* Calcd. for $C_{15}H_{22}N_2O_3 \cdot 1/2 H_2O$: C, 62.7; H, 8.07; N, 9.75. Found: C, 63.3; H, 7.95; N, 10.08. Evaporation of the original ether extracts gave a recovery of 0.40 g. (12%) of the TFA peptide ester.

10. *t*-Butyl Phthaloylglycylglycinate. A. By the Tetraethyl Pyrophosphite Procedure.—Phthaloylglycine[14] and *t*-butyl glycinate (0.020 mole of each) were treated with tetraethyl pyrophosphite (0.022 mole) in 14 ml. of dry dimethoxyethane by refluxing for 30 minutes. The product crystallized in quantitative yield on addition of 75 ml. of ice-water; m.p. 163–164°. Recrystallization from 75 ml. of absolute alcohol yielded 4.48 g. (92%), m.p. 165.5–166.5°.

B. By the Dicyclohexylcarbodiimide Procedure.—Phthaloylglycine, *t*-butylglycinate phosphite and triethylamine (0.010 mole of each) were dissolved in 17 ml. of tetrahydrofuran, then 2.27 g. (0.011 mole) of dicyclohexylcarbodiimide was added with stirring. The temperature of the solution slowly rose to 46°; after 5 minutes, the reaction was expedited by refluxing on a steam-bath for 10 minutes. The dicyclohexylurea which crystallized was collected at room temperature; m.p. 220–222°. One ml. of acetic acid was added to decompose excess dicyclohexylcarbodiimide, and 50 mg. of a precipitate having m.p. 153–156° was collected after 2 hours. Addition of 50 ml. of water to the filtrate gave a crystalline precipitate; weight 2.68 g., m.p. 145–148°. Solution of this product in 20 ml. of methyl ethyl ketone left 30 mg. of the urea undissolved. Addition of 50 ml. of *n*-heptane to the filtrate gave 1.0 g. of crystalline peptide derivative, m.p. 164–165° and additional heptane gave 0.35 g., m.p. 161–163°. The higher melting fraction was recrystallized from absolute ethanol; weight 0.8 g., m.p. 165–165.5°. *Anal.* Calcd. for $C_{16}H_{18}N_2O_5$: C, 60.4; H, 5.70; N, 8.80. Found: C, 60.3; H, 5.57; N, 8.93.

Evaporation of the original methyl ethyl ketone–heptane filtrates gave 0.89 g. of a compound having m.p. 150–160°. Recrystallization of this from absolute ethanol yielded 0.42 g., m.p. 180–182°, which gave correct analyses for **phthaloylglycyldicyclohexylurea.** Calcd. for $C_{23}H_{29}N_3O_4$: C, 67.13; H, 7.10; N, 10.21. Found: C, 67.11; H, 7.43; N, 10.15.

11. Phthaloylglycylglycine from its *t*-Butyl Ester.—The ester (0.74 g.) was added to a solution of 0.77 g. of hydrogen bromide in 5 ml. of glacial acetic acid at 10°. The material apparently did not dissolve, so an additional 4 ml. of acetic acid was added and hydrogen bromide was bubbled in for 2 minutes. Insoluble material was collected, washed with a few drops of acetic acid, then a few ml. of water, then dried. The product had m.p. 233–234°, and the m.p. of a sample mixed with authentic material[15] was not depressed. The yield was 0.58 g. (95%).

12. *t*-Butyl Benzyloxycarbonylprolyl-L-leucinate. A. By the Tetraethyl Pyrophosphite Method.—*t*-Butyl L-leucinate was liberated from its phosphite salt by treatment with aqueous alkali, extraction into ether, drying the solution with anhydr. sodium sulfate and evaporation of the ether under vacuum. The product from 2.16 g. (0.0080 mole) of the phosphite was dissolved in 20 ml. of redistilled diethyl phosphonate along with 1.99 g. (0.0080 mole) of benzyloxycarbonyl-L-proline[10] and 3.0 ml. (0.011 mole) of tetraethyl pyrophosphite. After heating 30 minutes on a steam-bath the solution was added to 50 ml. of ice-water and the product precipitated as an oil which soon crystallized. This was washed with 10 ml. of 5% potassium bicarbonate solution, then water (2 × 10 ml.). The air-dried material had m.p. 89–91°, wt. 3.3 g. (97% yield). A sample was recrystallized from heptane containing a small amount of isopropyl alcohol; m.p. 89–90°, $[\alpha]^{25}D$ −76.5° (*c* 5, MeOH). *Anal.* Calcd. for $C_{23}H_{34}N_2O_5 \cdot 1/2H_2O$: C, 64.6; H, 8.25; N, 6.56. Found: C, 64.6; H, 7.79; N, 6.67.

B. By the *p*-Nitrophenyl Ester Method.—A solution of 3.76 g. (0.010 mole) of benzyloxycarbonyl-L-proline *p*-nitrophenyl ester[16] and 1.87 g. (0.010 mole) of *t*-butyl L-leucinate in 15 ml. of methylene chloride was kept at room temperature for 48 hours. It was then washed with 30 ml. of 0.5 *N* sodium hydroxide solution (to remove *p*-nitrophenol) followed by 30 ml. of water. Washing with 15 ml. of an aqueous solution of phosphorous acid (1 g.) followed by 10 ml. of water (to remove any base) left a solution of the peptide derivative. This was obtained as a colorless solid, m.p. 89.5–91°, wt. 4.15 g. (96% yield as the hemihydrate), by evaporation of the solvent. A 1.00-g. sample was recrystallized from 15 ml. of heptane to yield 0.85 g., m.p. 91.5–92°.

13. Benzyloxycarbonyl-L-prolyl-L-leucine.—A solution of 1.07 g. (2.5 millimoles) of Z-pro-leu-O-*t*-Bu(L, L) and 0.10 g. of *p*-toluenesulfonic acid in 10 ml. of benzene was refluxed for an hour. The product was extracted with 30 ml. of 5% aqueous potassium bicarbonate solution. Acidification of the aqueous solution produced a crystalline precipitate of Z-pro-leu-OH (L, L), m.p. 117.5–118.5°, weight 0.38 g. Recrystallization from isopropyl alcohol (5 ml.) plus water (20 ml.) left 0.34 g. (37% yield), m.p. 118.5–119.5°, $[\alpha]^{25}D$ −62.7° (*c* 5, methanol). *Anal.* Calcd. for $C_{19}H_{26}N_2O_5$: C, 63.0; H, 7.23; N, 7.73. Found: C, 62.8; H, 7.44; N, 7.70.

Unreacted Z-pro-leu-O-*t*-Bu was recovered from the benzene solution; wt. 0.40 g. (37%), m.p. 91–92°.

14. *t*-Butyl *t*-Butyloxycarbonyl-L-prolyl-L-leucinate.—A solution of 4.30 g. (0.020 mole) of *t*-BOC-pro-OH(L),[17] 3.74 g. (0.020 mole) of H-leu-O-*t*-Bu (L) and 6 ml. of tetraethyl pyrophosphite in 10 ml. of dry dimethoxyethane was refluxed for 30 minutes. Addition of 30 ml. of water and chilling gave the crystalline product. This was collected and washed with 5% potassium bicarbonate, then water. After thorough drying in a vacuum desiccator, the yield was 6.97 g. (91%) and m.p. 93–94°.

Recrystallization from 50 ml. of ethanol by adding 60 ml. of water yielded 5.32 g., and addition of more water gave 0.86 g. which was recrystallized again to give 0.64 g.; total 5.96 g. (76%), m.p. 93–94°. A sample prepared by the same procedure had the same m.p. and $[\alpha]^{25}D$ −78.3° (*c* 5, methanol). This compound shows dimorphism; the melt at 94° soon crystallizes and this will then melt at 107–108°. The higher melting form readily goes over to the lower melting form on grinding with a spatula. *Anal.* Calcd. for $C_{24}N_{36}N_2O_5$: C, 62.5; H, 9.44; N, 7.29. Found: C, 62.4; H, 9.75; N, 7.37.

15. L-Prolyl-L-leucine. A. From Compound 12.—A saturated solution of hydrogen bromide in glacial acetic acid was made at 0°. Then 2.14 g. (5 millimoles) of Z-pro-leu-O-*t*-Bu(L,L) was added. Gas was rapidly evolved on warming to room temperature. After 20 minutes, the hydrobromide of H-pro-leu-OH was precipitated as a gum by the addition of about 100 ml. of anhydrous ether. This was separated, dissolved in 20 ml. of water and ammonium hydroxide was added to *p*H 7. The free peptide crystallized as a hemihydrate; dry weight 0.79 g. (67%), m.p. 247–249°. Recrystallization from 80 ml. of water yielded 0.40 g., m.p. 249.5–250.5°, and addition of an equal volume of dimethoxyethane to the filtrate yielded 0.10 g. with the same m.p.; total yield 42%, $[\alpha]^{25}D$ −79° (*c* 0.18, water). *Anal.* Calcd. for $C_{11}H_{20}N_2O_3 \cdot 1/2H_2O$: C, 55.7; H, 8.92; N, 11.8. Found: C, 55.9; H, 8.94; N, 12.0.

B. From Compound 14.—*t*-BOC-pro-leu-O-*t*-Bu(2L) was treated with hydrogen bromide in acetic acid by the same procedure used in A. The crude yield of H-pro-leu-OH(2L) was 78% and the recrystallized yield (from water, in which the peptide is somewhat soluble) was 39%, $[\alpha]^{25}D$ −58 ± 1.5° (*c* 0.17, water), m.p. 250°. The discrepancy in rotation compared to the product in A is being investigated.

(14) J. H. Billman and W. F. Harting, THIS JOURNAL, **70**, 1473 (1948).

(15) J. C. Sheehan and V. S. Frank, *ibid.*, **71**, 1856 (1949).

(16) M. Goodman and K. C. Steuben, *ibid.*, **81**, 3980 (1959).

(17) G. W. Anderson and A. C. McGregor, *ibid.*, **79**, 6180 (1957).

16. *t*-Butyl Trifluoroacetylglycyl-L-prolinate.—Trifluoroacetylglycine[18] (1.71 g., 0.010 mole), *t*-butyl L-prolinate (1.71 g., 0.010 mole) and tetraethyl pyrophosphite (3 ml., 0.011 mole) were added to 7 ml. of dry dimethoxyethane. After 30 minutes of refluxing, the solution was added to 50 ml. of ice-water and the peptide derivative soon crystallized. It was collected, washed with water and air-dried; wt. 2.27 g. (69%), m.p. 89–90°. A 1-g. sample recrystallized from 10 ml. of methylcyclohexane yielded 0.85 g. having the same m.p. and $[\alpha]^{25}D$ −83° (*c* 2, ethanol). *Anal.* Calcd. for $C_{13}H_{19}F_3N_2O_4$: C, 48.1; H, 5.91; N, 8.64; F, 17.6. Found: C, 48.2; H, 5.78; N, 8.90; F, 17.8.

17. Ethyl Glycyl-DL-phenylalaninate. (Table IV).—Ethyl benzyloxycarbonylglycyl-DL-phenylalaninate[3] (3.84 g.) was added to 20 ml. of a solution of *N* hydrogen bromide in glacial acetic acid and the resulting solution was heated on a steam-bath for 15 minutes. Addition of a double volume of ether and chilling caused crystallization of 3.05 g. (93% yield) of **ethyl glycyl-DL-phenylalaninate hydrobromide**, m.p. 154–155°; calcd. 24.1% Br, found 24.0% (Volhard titration). The free base was obtained as an oil by adding anhydrous ammonia to a suspension of 1.66 g. (0.0050 mole) in 75 ml. of anhydrous ether, filtering off the precipitated ammonium bromide (Celite used) and evaporating the ether solution. The recovery was 90.4% for the dipeptide ester. Crystallization began in 38 minutes. After 5 hours at 25° the product was triturated with anhydrous ether (3 × 10 ml.) and air-dried; a 73% yield (670 mg.) of DL-3(6)-benzyl-2,5-diketopiperazine, m.p. 271–274° dec., was obtained. After a period of 23 hours an additional 150 mg. of diketopiperazine with the same m.p. was isolated, making a total recovery of 89% from the free base.

Comparative Stabilities of *t*-Butyl Glycinate and Ethyl Glycinate. (Table IV).—The free base of *t*-butyl glycinate was prepared by shaking 0.050 mole (10.65 g.) of the phosphite salt with a mixture of 50 ml. of 2 *N* sodium hydroxide and 100 ml. of ether. The ether layer was separated, washed with 10 ml. of water and dried with anhydrous sodium sulfate. Concentration of the solution and distillation of the residue at 2 mm. yielded 4.0 g. (62%), b.p. 30°, $n^{24}D$ 1.4227. The distillate was divided into two equal portions and each was placed in a screw-cap vial to exclude moisture and carbon dioxide.[19] One sample was kept at −20° and the other at room temperature. Freshly prepared ethyl glycinate was prepared and kept under identical conditions for comparison. The latter solidified completely during 4 days at room temperature, and was about one-third solid after 21 days at −20°. By contrast, *t*-butyl glycinate was unchanged after 30 days and only about one-fourth solidified after 325 days at room temperature; at −20°, no solid had formed in 700 days and the refractive index was unchanged. Reconversion to the phosphite salt, m.p. 157–159° dec., was quantitative.

TABLE IV

STABILITY OF AMINO ACID AND DIPEPTIDE ESTERS AS FREE BASES

	Amino ester	Solid formation,[a] % At rm. temp.	At −20°
1	H·gly·OEt[b]	100/4 days	35/21 d.
2	H·gly·O-*t*-Bu	25/325 d.	0/700 d.
3	H·pro·O-*t*-Bu(L)	Trace/150 d.	
4	H·pro·OEt(L)[c]	100/30 d.	
5	H·gly-phe·OEt(DL)	73/5 hr.	
6	H·gly-phe·O-*t*-Bu(L)	0/23 d.	Trace/540 d.

[a] The ether-insoluble solid from (5) was found to have the m.p. of the known diketopiperazine (see Exptl.). The trace of material from (6) had m.p. 95° dec., whereas the reported m.p. of the diketopiperazine is 260° (H. T. Huang and C. Niemann, THIS JOURNAL, **72**, 921 (1950)). [b] Prepared from the hydrochloride by NH_3 in ether and distilled under red ced pressure. [c] Prepared from the hydrochloride by Et_3N in ether; b.p. 53° at 2.5 mm., $n^{16}D$ 1.4500.

(18) F. Weygand and E. Leising, *Ber.*, **87**, 248 (1954).

(19) K. T. Poroshin, Yu. I. Khurgin and T. D. Kozarenko, *Bull. Acad. Sci. U.S.S.R.*, 1453 (1959), have shown that carbon dioxide catalyzes the self-condensation of ethyl glycinate.

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN CO.]

The Synthesis of Nucleoside and Nucleotide Analogs Derived from Uridine*

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The synthesis of a diethyl 5′-deoxy-5′-phosphonate analog of uridylic acid is described, together with certain other 5′-substituted uridines. The 5′-deoxy-5′-mercapto analog of uridine exists in the solid state and in neutral and acid solutions as a 5,6-dihydro-5′,6-cyclic sulfide. In alkaline solution (0.01 *N*), ring opening occurs to regenerate the 5,6-double bond of an authentic uridine structure together with the formation of the 5′-mercaptide anion (*vide* XIII → XVI).

The importance of uridylic acid in ribonucleic acid synthesis and its determination as a constituent of the novel nucleotide shown by Park and Strominger[1] to be implicated in bacterial cell wall formation made it of interest to examine analogs of uridylic acid as potential antitumor, antiviral and antibacterial agents.

Although considerable data have been accumulated concerning nuclear derivatives of pyrimidine nucleosides,[2] relatively little information is available concerning pyrimidine nucleosides which incorporate substituted sugar moieties.[3] For this reason, attention in the present work was directed initially toward the synthesis of the phosphonic acid analog (V) of uridylic acid (VI). The phosphonic acid group bears an extremely close resemblance to the acid function of a monophosphate ester both sterically and in pK_a values, as has been pointed out previously.[2,4,5]

Burger, *et al.*,[6] have described the syntheses of "7-[2,3,4-tri-O-acetyl-6-deoxy-6-(diethyl phosphonate)-β-D-glucopyranosyl]-theophylline" and "6-benzamido-9-[2,3,4-tri-O-acetyl-6-deoxy-6-(diethyl phosphonate)-β-D-glucopyranosyl]-purine" in continuation of studies of phosphonate analogs of nucleotides, but no phosphonate analog of a naturally occurring nucleoside phosphate has been re-

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(1) J. T. Park and J. L. Strominger, *Science*, **125**, 99 (1957).

(2) See, *inter alia*, "Antimetabolites of Nucleic Acid Precursors," D. W. Visser in "Antimetabolites and Cancer," A.A.A.S. Symposium, A.A.A.S. publication, 1955, p. 47.

(3) J. J. Fox, J. F. Codington, N. C. Yung, L. Kaplan and J. O. Lampen, THIS JOURNAL, **80**, 5155 (1958), and earlier papers.

(4) S. Preis, T. C. Myers and E. V. Jensen, THIS JOURNAL, **77**, 6225 (1955).

(5) J. R. Parikh and A. Burger, *ibid.*, **77**, 2386 (1955); B. S. Griffin and A. Burger, *ibid.*, **78**, 2336 (1956).

(6) J. R. Parikh, M. E. Wolff and A. Burger, *ibid.*, **79**, 2778 (1957).