the added stability required by the calculated rate constant for the B chains. The bond in the A chains between leucine and the third amino acid residue is certainly a labile one despite the fact that leucine is involved. Whether or not this bond is an unusually labile one cannot be decided because investigations of the kinetics of peptide hydrolysis usually are made with milder conditions than refluxing 6 *N* hydrochloric acid; however, a bond such as leu-ser or leu-thr probably would be rather labile, even though leucine is involved, because of the unusual lability of peptide bonds that involve the amino group of serine or threonine.[16]

Finally, then, the results of the present investigation lead to the following conclusions. Normal adult human hemoglobin contains 4 N-terminal valyl residues per molecule. Upon hydrolysis of DNP-globin in refluxing 6 *N* hydrochloric acid, two of these residues are released within 15 min. as DNP-val-leu. The other two residues have not been detected with certainty in any form other than DNP-valine. Whether hemoglobin contains branched or unbranched chains, our results and the fact that the C-terminal residues are unlike require that hemoglobin contain at least two kinds of polypeptide chains. On the basis of his examination of tryptic hydrolyzates of hemoglobin, Ingram[22] has concluded that hemoglobin contains identical half molecules, a conclusion that has also been drawn from the X-ray investigations of Perutz and his collaborators.[23] Ingram also concludes that four identical sub-units are not present.

Acknowledgments.—This investigation was supported in part by a grant from the Ford Foundation and in part by a research grant (RG-4276(C)) from the National Institutes of Health, Public Health Service.

(22) V. M. Ingram, *Nature*, **178**, 792 (1956).

(23) M. F. Perutz, A. M. Liquori and F. Eirich, *ibid.*, **167**, 929 (1951).

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[CONTRIBUTION FROM THE STERLING-WINTHROP RESEARCH INSTITUTE]

New Amine-masking Groups for Peptide Synthesis

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RECEIVED MARCH 30, 1957

Acid-catalyzed cleavage of a urethan, R'NHCOOR, leads to the formation of an amine salt, carbon dioxide and a product derived from the carbonium ion, R^+. This paper reports the use in peptide synthesis of urethans derived from *t*-butyl alcohol, *p*-methoxybenzyl alcohol, cyclopentanol, cyclohexanol and diisopropylcarbinol. Some of these carboalkoxy protecting groups possess advantages over the well-known carbobenzoxy group.

It has been pointed out that the cleavage of a carbobenzoxy group from an amino acid or peptide by phosphonium iodide is an acid-catalyzed reaction,[1,2] and in recent years there have been a number of reports of the use of the more convenient anhydrous hydrogen halides in place of phosphonium iodide.[1-6]

The reaction may be written as $ROCONHR' + 2H^+ \rightarrow R^+ + CO_2 + H_3N^+R'$, where R is benzyl and R' is an amino acid or peptide residue. There are, however, sometimes objections to the use of benzyl (or allyl) groups. For example, benzyl iodide and benzyl bromide are lachrymators. In addition, the benzyl ion attacks the sulfur atom in methionine,[2] and peptides of tryptophan are frequently obtained in poor yield or no yield at all. It is also sometimes desirable to have a protecting group which would be stable to catalytic hydrogenation. Thus a benzyl ester could be unmasked while leaving the amine protected.

It already has been shown[2] that carboisopropoxyglycyl-DL-phenylalanine gives a diketopiperazine when treated with hydrogen bromide in nitromethane. Here the cleavage of the urethan is so slow that a competing reaction becomes of major importance. Thus, a carbonium ion more stable than the isopropyl is required. During 1952, we undertook the investigation of the use of other urethans as amine-masking groups; the results form the subject of the present paper.

An advantage of the carbo-*t*-butoxy group as a masking agent is that it is cleaved with extreme ease. Carbon dioxide evolution begins immediately when hydrogen chloride is bubbled into a solution of a carbo-*t*-butoxypeptide; the reaction is more rapid than the removal of a carbobenzoxy group with hydrogen bromide. It has been found that refluxing acetic acid will also liberate carbon dioxide from a carbo-*t*-butoxy tripeptide, whereas the carbobenzoxy group is stable under these conditions. However, the product is a diketopiperazine and not a peptide when acetic acid is used for the decomposition. It was suspected that the peptide was the first product and that this cyclized to a diketopiperazine and a free amino acid on further heating in acetic acid. The literature affords several examples of reactions of this type. Lichtenstein heated, di-, tri- and tetrapeptides in β-naphthol at 135–150° and obtained diketopiperazines (and a free amino acid in the case of tripeptides).[7] Emerson has pointed out that prolonged heating with hydrazine in the removal of the phthaloyl protecting group will lead to diketopipera-

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

(2) N. Albertson and F. McKay, THIS JOURNAL, **75**, 5323 (1953).

(3) E. Waldschmidt-Leitz and K. Kuhn, *Ber.*, **84**, 381 (1951).

(4) G. Anderson, J. Blodinger and A. Welcher, THIS JOURNAL, **74**, 5309 (1952).

(5) R. Boissonnas and G. Preitner, *Helv. Chim. Acta*., **35**, 2240 (1952); **36**, 875 (1953).

(6) D. Ben-Ishai, *J. Org. Chem.*, **19**, 62 (1954).

(7) N. Lichtenstein, THIS JOURNAL, **60**, 560 (1938).

TABLE I[a]

Peptide or amide	Yield, %	M.p., °C.	Formula	N(AP) Calcd.	N(AP) Found	N(K) Calcd.	N(K) Found
From carbo-*t*-butoxy derivatives							
Glycyl-DL-methionine	92	203–205d	$C_7H_{14}N_2O_3S$	6.79	6.66	13.58	13.38
Glycyl-DL-tryptophan	52	231–233d	$C_{13}H_{15}N_3O_3$	5.36	5.20	16.08	15.62
Glycyl-DL-phenylalanine	40	272–273d	$C_{11}H_{14}N_2O_3$	6.31	6.03	12.62	12.46
DL-Methionylglycine	80	213–215d	$C_7H_{14}N_2O_3S$	6.79	6.60	13.58	13.24
DL-Phenylalanylglycine	91	273–275d	$C_{11}H_{14}N_2O_3$	6.31	6.25	12.62	12.49
DL-Phenylalanyl-β-alanine	38	200–207d	$C_{12}H_{16}N_2O_3$	5.93	5.77	11.85	11.75
DL-Valylglycine	95	247d	$C_7H_{14}N_2O_3$	8.04	7.84	16.08	15.95
DL-Valine amide·HBr[b]	66	238–240d	$C_5H_{12}N_2O\cdot HBr$				
L-Leucyl-L-leucine[c]	17	270–272d	$C_{12}H_{24}N_2O_3$	5.73	5.41	11.47	11.22
L-Leucine amide·HBr[d]	94	235–238d	$C_6H_{14}N_2O\cdot HBr$			13.28	13.58
DL-Alanylglycine	91	224–225d	$C_5H_{10}N_2O_3$	9.59	9.42	19.17	19.22
From carbocyclopentyloxy derivatives							
Glycyl-DL-phenylalanine	73	273–275d	$C_{11}H_{14}N_2O_3$	6.31	6.04	12.62	12.55
DL-Valylglycine	60	247d	$C_7H_{14}N_2O_3$	8.04	7.92	16.08	16.04
L-Valyl-L-leucylglycine	56	237–240d	$C_{13}H_{25}N_3O_4$	4.87	4.67	14.62	14.38
DL-Alanylglycine	80	229–231d	$C_5H_{10}N_2O_3$	9.59	9.50	19.17	18.94
β-Alanyl-DL-phenylalanine	71	253–255d	$C_{12}H_{16}N_2O_3$	5.93	5.68	11.85	11.87
DL-Prolylglycine	69	232–236d	$C_7H_{12}N_2O_3$	8.13	8.02		
L-Isoleucyl-L-asparagine[e]	57	231–236	$C_{10}H_{19}N_3O_4$	5.71	5.73	17.13	17.22
From carbo-*p*-methoxybenzyloxy derivatives							
DL-Methionylglycine	45	210–212d	$C_7H_{14}N_2O_3S$	6.79	6.58	13.58	13.30
Glycyl-DL-methionine	36	202–204d	$C_7H_{14}N_2O_3S$	6.79	6.65	13.58	13.50
From carbocyclohexyloxy derivatives							
Glycylglycine	55	>250	$C_4H_8N_2O_3$	10.60	10.48		
Glycyl-L-leucylglycine[f]	..	214–215	$C_{10}H_{19}N_3O_4$	5.71	5.69		
DL-Phenylalanylglycine	42	271–274[g]	$C_{11}H_{14}N_2O_3$	6.30	5.85[g]		
DL-Phenylalanyl-β-alanine	18	206–208	$C_{12}H_{16}N_2O_3$	6.25	5.93	12.49	12.00
From carbo-(diisopropyl)-methoxy derivatives							
Glycyl-DL-phenylalanine	36	273–275d	$C_{11}H_{14}N_2O_3$	6.31	5.82	12.62	12.39

[a] N(AP) refers to nitrogen determined by titration in acetic acid with perchloric acid; N(K) refers to Kjeldahl nitrogen. [b] Br^- calcd., 41.06; Br^- found, 40.50. [c] $[\alpha]^{24}D$ −13.4° (8.1% in normal sodium hydroxide). The same rotation was reported by Fischer, *Ber.*, **39**, 2893 (1906). [d] Br^- calcd., 37.88; Br^- found 37.50; $[\alpha]^{25}D$ +8.72° (5% in water). [e] $[\alpha]^{25}D$ +21.2° (5% in water). [f] $[\alpha]^{25}D$ −43.3° (2.46% in water). M. Bergmann, L. Zervas and J. Fruton, *J. Biol. Chem.*, 111, 225 (1935), report a rotation of −41.2°. [g] Value for crude product.

zine formation.[8] This type of reaction may possibly have some application in determining the amino acid sequence of peptides.[9]

One advantage to the carbo-*t*-butoxy group is that there is no attack of the sulfur atom of methionine by the intermediate carbonium ion (presumably because stabilization is achieved by expulsion of a proton). Thus, for peptides containing methionine the carbo-*t*-butoxy group may offer some advantages over the carbobenzoxy group. The carbo-*t*-butoxy group also appears to be satisfactory for peptides containing tryptophan, whereas the carbobenzoxy group is generally unsatisfactory when acid cleavage is used.

The principal disadvantage to the use of the carbo-*t*-butoxy group lies in the difficulty of preparing and storing carbo-*t*-butoxy chloride.[10] However, amino acid esters are converted readily to isocyanates with phosgene[11] and these will react with *t*-butyl alcohol to form carbo-*t*-butoxyamino acid esters. Saponification gives the carbo-*t*-butoxyamino acid.

A second approach to carbo-*t*-butoxyamino acids is based on the instability of alkyl-aryl carbonates to base.[12] Thus, by using a mixed carbonate one should be able to replace the aryloxy group by an amine according to the equation

$$(CH_3)_3COC(=O)OC_6H_5 + NH_2CHRCOOEt \longrightarrow (CH_3)_3COCONHCHRCOOEt + C_6H_5OH$$

Although this reaction went as anticipated, the yields were poor. Even the carboisopropoxy and carboisobutoxy groups were introduced in yields of only 20–30%. Only a trace of products was obtained from the reaction of amino acid esters with the mixed carbonate obtained by using thiophenol in place of phenol.

Transesterification using *t*-butyl alcohol and a carbophenoxyamino acid ester unexpectedly failed to give the desired product.

(8) O. H. Emerson, U. S. Patent 2,498,665.

(9) For example, the structure of an octapeptide containing eight different amino acids would be uniquely determined if one could identify the four diketopiperazines obtained on initial degradation, remove the first amino acid by known methods, and identify the three diketopiperazines obtained by degradation of the resulting heptapeptide. It is also obvious that the diketopiperazines which would be formed first would be near the amino end of the peptide assuming that degradation proceeded from the amino end.

(10) A. Choppin and J. Rogers, THIS JOURNAL, **70**, 2967 (1948); *cf.* also German Patent 254,471 for the preparation of the chloroformate of dimethylethylcarbinol.

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

(12) *Cf.* P. Cazeneuve, *Bull. soc. chim.*, [3] **25**, 634 (1901).

TABLE II

	Amino acid	Yield, %	M.p., °C.	Formula	Nitrogen, % Calcd.	Found	Neut. equiv. Calcd.	Found
Carbo-*t*-butoxy[a]	Glycine	56	85–89	$C_7H_{13}NO_4$	7.99	7.98	175	177
	DL-Alanine	55	103–106	$C_8H_{15}NO_4$	7.40	7.60	189	184
	DL-Methionine	80	87–91	$C_{10}H_{19}NO_4S$	5.62	5.72	249	252
	L-Leucine	72	74–80	$C_{11}H_{21}NO_4$	6.06	5.64		
Carbocyclopentyloxy[b]	Glycine	69	77–80	$C_8H_{13}NO_4$	7.48	7.44	187	190
	L-Asparagine	66	177–179	$C_{10}H_{16}N_2O_5$	11.47	11.51	244	245
	DL-Valine	75	100–102	$C_{11}H_{19}NO_4$	6.11	6.18	229	228
	DL-Alanine	74	120–123	$C_9H_{15}NO_4$	6.96	7.02	201	200
	β-Alanine	66	54–58	$C_9H_{15}NO_4$	6.96	7.00	201	199
	DL-Methionine	71	111–113	$C_{11}H_{19}NO_4S$	5.36	5.38	261	258
	γ-Ethyl L-glutamate	64	62–66	$C_{13}H_{21}NO_6$	4.88	4.95	287	288
	2-Phenylglycine	83	93–95	$C_{14}H_{17}NO_4$	5.32	5.39	263	263
	DL-Serine	62	118–119	$C_9H_{15}NO_5$	6.44	6.32	217	219
	L-Phenylalanine	58	123–127	$C_{15}H_{19}NO_4$	5.05	4.97		
	DL-Isoleucine	82	95–99	$C_{12}H_{21}NO_4$	5.76	5.77	243	245
	DL-Norleucine	69	98–102	$C_{12}H_{21}NO_4$	5.76	5.77	243	245
	DL-2-Aminopelargonic acid	87	99–101	$C_{15}H_{27}NO_4$	4.91	4.73	285	287
α,ε-Dicarbocyclopentyloxy	L-Lysine	75	93–100	$C_{18}H_{30}N_2O_6$	7.56	7.35	370	375
Carbocyclohexyloxy[c]	Glycine	80	97–99	$C_9H_{15}NO_4$	6.96	7.03	201	207
	DL-Alanine	41	125–126	$C_{10}H_{17}NO_4$	6.51	6.47	215	215
	DL-Methionine	60	97–100	$C_{12}H_{21}NO_4S$	5.05	5.05	275	277
	DL-Phenylalanine	82	105–108	$C_{16}H_{21}NO_4$	4.81	4.70	291	297
Carbo-*p*-methoxybenzyloxy[d]	Glycine	38	94–97	$C_{11}H_{13}NO_5$	5.85	5.92	239	246
Carbo-(diisopropyl)-methoxy	Glycine	76	80–83	$C_{10}H_{19}NO_4$	6.45	6.44	217	218

[a] The DL-phenylalanine and DL-valine derivatives were sirups. [b] Derivatives of L-leucine, L-valine and DL-proline were sirups; L-isoleucine gave large hexagonal prisms on standing. [c] Carbocyclohexyloxy-L-leucine was a waxy solid. [d] Carbo-*p*-methoxybenzyloxy-DL-methionine was a sirup.

Remarks concerning the preparation and cleavage of the carbo-*t*-butoxy group apply for the most part to the carbo-*p*-methoxybenzyloxy group. It offers no particular advantge over the carbobenzoxy group.

At this point it was felt that some group intermediate in activity between the isopropyl and *t*-butyl group might prove to be both easy to make and easy to remove, and also not be lachrymatory. The cyclopentyl group proved to be the best of the groups tried.

Cyclopentyl chloroformate is readily prepared in quantity from cyclopentanol and phosgene.[13] It can be distilled (although this is unnecessary) and may be kept for several months in a refrigerator.[14]

The preparation of carbocyclopentyloxyamino acids and peptides parallels the preparation of the corresponding carbobenzoxy derivatives. The removal of the carbocyclopentyloxy group using hydrogen bromide in nitromethane takes a little longer than the removal of the carbobenzoxy group, but the reaction is still rapid (less than 15 minutes).

The carbocyclohexyloxy and carbo-(diisopropyl)-methoxy protecting groups are removed less readily than the carbocyclopentyloxy group. Since slow removal of the urethan-protecting group allows a competing reaction to become of more importance,[2] work with the chloroformates of cyclohexanol and diisopropylcarbinol was discontinued when it became apparent that the experimental results were bearing out the superiority of the carbocyclopentyloxy group.

For some purposes the carbocyclopentyloxy group would have the advantage of not being cleaved by hydrogenolysis, thus affording a distinction from the carbobenzoxy group. Since the products of acid-catalyzed cleavage of the carbocyclopentyloxy group are not lachrymatory, the carbocyclopentyloxy intermediates are more pleasant to work with than the carbobenzoxy intermediates.

Typical examples of peptides prepared from these new carboalkoxy protecting groups are shown in Table I. The yields reported in the tables are probably not the maximum yields to be expected since mother liquors were never worked up. All of the peptides shown in Table I were prepared by cleavage with hydrogen bromide except peptides containing tryptophan or methionine; hydrogen chloride was used in these cases. Examples of the simultaneous removal of both the amine and the carboxyl masking groups as well as an example of the preferential removal of a carbo-*t*-butoxy group are given in the Experimental section.

Experimental

Alkyl Chlorocarbonates.—Phosgene was condensed at the temperature of a Dry Ice–acetone-bath until a 50% excess has been collected. The appropriate alcohol was then dripped into the liquid phosgene at 0°. The mixture was allowed to stand 4 hours at room temperature and the excess phosgene was removed *in vacuo* at room temperature. Yields were above 90% on a 4-mole scale. The chlorocarbonates were used, without distillation, to prepare carbocyclopentyloxy-, carbocyclohexyloxy- and carbo-(diisopropyl)-methoxyamino acids listed in Table II.

(13) A modified preparation of cyclopentyl chloroformate was recently reported by S. Nakanishi, T. Myers and E. Jensen, THIS JOURNAL, **77**, 3099 (1955).

(14) A purified sample of cyclohexyl chloroformate blew up after approximately two months in a sealed ampule at room temperature. The precautions used with benzyl chloroformate should also be observed with the chloroformates mentioned in this paper.

TABLE III

Ester or amide	Yield, %	M.p., °C.	Formula	Nitrogen, % Calcd.	Found
C-*t*-BuO-Gly-DL-Phe-OMe[a]	78	89–92	$C_{17}H_{24}N_2O_5$	8.33	8.08
C-*t*-BuO-DL-Phe-Gly-OMe	45	150–152	$C_{17}H_{24}N_2O_5$	8.33	8.32
C-*t*-BuO-DL-Phe-β-Ala-OEt	35	102–106	$C_{19}H_{28}N_2O_5$	7.68	7.81
C-*t*-BuO-DL-Ala-Gly-OMe	50	101–102	$C_{11}H_{20}N_2O_5$	10.76	10.75
C-*t*-BuO-Val-Gly-OMe	72	113–114	$C_{13}H_{24}N_2O_5$	9.71	9.77
C-*t*-BuO-DL-Val-NH_2	39	146–148	$C_{10}H_{20}N_2O_3$	12.94	13.18
C-*t*-BuO-L-Leu-L-Leu-OEt	48	130–133	$C_{19}H_{36}N_2O_5$	7.52	7.58
C-*t*-BuO-L-Leu-NH_2	54	136–140	$C_{11}H_{22}N_2O_3$	12.17	12.10
C-*t*-BuO-L-Leu-Gly-OMe	80	128–131	$C_{15}H_{26}N_2O_5$	9.26	9.33
CCpO-Gly-DL-Phe-OMe[b]	61	104–105	$C_{18}H_{24}N_2O_5$	8.03	8.07
CCpO-β-Ala-DL-Phe-OMe	86	102–105	$C_{19}H_{26}N_2O_5$	7.73	7.68
CCpO-DL-Ala-Gly-OMe	68	84–87	$C_{12}H_{20}N_2O_5$	10.29	10.33
CCpO-DL-Val-Gly-OMe	68	135–136	$C_{14}H_{24}N_2O_5$	9.32	9.46
CCpO-L-Leu-L-Leu-OEt	64	139–140	$C_{20}H_{36}N_2O_5$	7.28	7.33
CCpO-L-Val-L-Leu-Gly-OMe	88[c]	172–177[c]	$C_{20}H_{35}N_3O_6$	10.16	10.47[c]
CCpO-DL-Met-Gly-OMe	71	78–100	$C_{14}H_{24}N_2O_5S$	8.43	8.38
CCpO-L-Val-Gly-OMe	68	149–154	$C_{14}H_{24}N_2O_5$	9.32	8.94
CCpO-DL-Norleu-Gly-OCp[d]	69	101–102	$C_{19}H_{32}N_2O_5$	7.60	7.61
CCpO-L-Ileu-Gly-OMe	52	144–146	$C_{16}H_{26}N_2O_5$	8.91	8.96
CCpO-DL-Norval-Gly-OMe	66	93–95	$C_{14}H_{24}N_2O_5$	9.33	9.29
CCpO-DL-Norval-Gly-OCp	63	88–90	$C_{18}H_{30}N_2O_5$	7.91	7.96
CCpO-L-Val-L-Ser-OMe	44	149–155	$C_{16}H_{26}N_2O_6$	8.48	8.25
CCpO-L-Asp(NH_2)-L-Glu(OCp)$_2$[e]		163–167	$C_{25}H_{39}N_3O_8$	8.25	8.15
CCpO-O-Ac-L-Tyr-Gly-OMe	57	143–147	$C_{20}H_{26}N_2O_7$	6.88	6.75
CCpO-DL-2-Aminophenylacetyl-Gly-OCp	84	118–122	$C_{21}H_{28}N_2O_5$	7.21	7.17
CCpO-L-Leu-Gly-OCp	70	81–82	$C_{19}H_{32}N_2O_5$	7.59	7.57
CCpO-Gly-Gly-OMe	62	67–70	$C_{11}H_{18}N_2O_5$	10.85	10.82
CCpO-6-Aminocaproyl-Gly-OCp	76	72–75	$C_{21}H_{30}N_2O_5$	7.18	7.18
CCpO-L-Val-Gly-OCp	77	132–134	$C_{18}H_{30}N_2O_5$	7.91	7.79
CCpO-Gly-L-Leu-Gly-OCp[f]	63	124–128	$C_{21}H_{35}N_3O_6$	9.88	9.95
CCpO-DL-Ileu-DL-Val-OMe	61	100–111	$C_{18}H_{32}N_2O_5$	7.86	7.74
CCpO-DL-Ileu-DL-Ser-OMe	63	100–107	$C_{16}H_{28}N_2O_6$	8.13	8.00
CCpO-L-Ileu-L-His-OMe	44	199–203	$C_{19}H_{30}N_4O_5$	14.21	13.50
CChO-DL-Phe-Gly-OMe[g]	71	113–115	$C_{19}H_{26}N_2O_5$	7.73	7.56
CChO-Gly-L-Leu-Gly-OMe	..	158–159	$C_{18}H_{31}N_3O_6$	10.90	10.68

[a] Carbo-*t*-butoxyglycyl-DL-phenylalanine methyl ester. This is essentially the system of abbreviations devised by B. Erlanger and E. Brand, THIS JOURNAL, **73**, 3509 (1951). [b] CCpO represents carbocyclopentyloxy. [c] Value for crude product. [d] Carbocyclopentyloxy-DL-norleucylglycine cyclopentyl ester. [e] Carbocyclopentyloxy-L-asparaginyl-L-glutamic acid dicyclopentyl ester. [f] From carbocyclopentyloxyglycine and the dipeptide ester. [g] CChO represents carbocyclohexyloxy.

Carbo-*t*-butoxy- and Carbo-*p*-methoxybenzyloxyamino Acids.—Isocyanates were prepared from amino acid esters by passing phosgene into a suspension of the ester hydrochlorides in refluxing toluene until all material had dissolved (3 to 7 hours).[11,15] Phosgene and toluene were then removed *in vacuo* and the crude isocyanates allowed to react with the appropriate alcohol for 15 minutes at steam-bath temperature. After standing 1 hour at room temperature, the esters were saponified. It was necessary to extract carbo-*t*-butoxyglycine from aqueous solutions with ether. Products were recrystallized from ethyl acetate. Carbo-*t*-butoxy- and carbo-*p*-methoxybenzyloxyamino acids are listed in Table II.

Carboalkyloxy peptide esters were prepared by the method of Vaughan, Boissonnas, and Wieland and Bernhard.[16] The esters and acids prepared by their saponifications are listed in Tables III and IV, respectively.

Peptides from carboalkyloxy peptides were prepared by treatment of the carboalkyloxy peptides with hydrogen bromide or hydrogen chloride in nitromethane as described in reference 2. The carbo-(diisopropyl)-methoxy group was removed after 3 days treatment at room temperature. Reaction was complete in the case of carbo-*t*-butoxy peptides in about one minute. Carbocyclopentyloxy peptides were cleaved in 4 to 8 minutes when hydrogen bromide was used.

Peptides from Carbocyclopentyloxy Dipeptide Cyclopentyl Esters.—The three peptides listed illustrate the simultaneous removal of a carbocyclopentyloxy masking group and a cyclopentyl ester group by treating overnight (about 15 hours) at room temperature with anhydrous hydrogen bromide in anhydrous acetic acid:

Glycyl-DL-phenylalanine: m.p. 270–271°, 68% yield. *Anal.* Calcd. for $C_{11}H_{14}N_2O_3$: N(AP), 6.31; N (total), 12.62. Found: N(AP), 6.02; N(K), 12.45.

DL-2-Aminophenylacetylglycine: m.p. 241–242°, 88% yield. *Anal.* Calcd. for $C_{10}H_{12}N_2O_3$: N(AP), 6.73; N(total), 13.46. Found: N(AP), 6.78; N(K), 13.24.

DL-Norleucylglycine: m.p. 225–227°, 78% yield. *Anal.* Calcd. for $C_8H_{16}N_2O_3$: N(AP), 7.44. Found: N(AP), 7.39.

Preferential Removal of the Carbo-*t*-butoxy Group.—The following example illustrates the removal of a carbo-*t*-butoxy group while leaving a carbobenzoxy group intact.

To 14.1 g. of α-carbo-*t*-butoxyglycyl-L-leucyl-ε-carbobenzoxy-L-lysine hydrazide was added 50 ml. of acetic acid containing 2.4 g. of dissolved hydrogen chloride. There was immediate evolution of carbon dioxide. The reaction vessel was cooled occasionally to keep the temperature of the reaction mixture below 30°. After 20 minutes, diethyl ether was added to the mixture to precipitate the product. There was thus obtained 12.8 g. (95% yield) of glycyl-L-leucyl-ε-carbobenzoxy-L-lysine hydrazide dihydrochloride as white crystals which melted at 115°.

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TABLE IV

Acid	Yield, %	M.p., °C.	Nitrogen, % Calcd.	Found	Neut. equiv. Calcd.	Found
C-*t*-BuO-Gly-DL-Met-OH[a]	51	138–140	9.14	9.09	306	310
C-*t*-BuO-Gly-DL-Phe-OH	55	128–131	8.68	8.74		
C-*t*-BuO-Gly-DL-Try-OH	48	157–159	11.63	11.60	361	363
C-*t*-BuO-DL-Phe-Gly-OH	66	180–181	8.68	8.64	322	328
C-*t*-BuO-DL-Phe-β-Ala-OH	86	173–175	8.33	8.29		
C-*t*-BuO-DL-Met-Gly-OH	71	138–141	9.14	9.27	306	306
C-*t*-BuO-DL-Ala-Gly-OH	72	168–170	11.37	11.29	246	248
C-*t*-BuO-DL-Val-Gly-OH	24	132–135	10.21	10.32		
C-*t*-BuO-L-Leu-L-Leu-OH	68	153–155	8.13	8.07		
C-*t*-BuO-Gly-DL-Met-OH	51	138–140	9.14	9.09	306	310
C-*t*-BuO-Gly-L-Leu-OH	71	112–116			288	274
CCpO-Gly-DL-Phe-OH[a]	89	129–131	8.37	8.20	335	334
CCpO-DL-Val-Gly-OH	75	155–158	9.77	9.83	286	285
CCpO-L-Val-L-Leu-Gly-OH	82[b]	180–189[b]	10.52	10.49[b]		
CCpO-DL-Ala-Gly-OH	77	158–159	10.83	10.92	258	255
CCpO-β-Ala-DL-Phe-OH	78	140–145	8.03	8.09	348	348
CCpO-DL-Pro-Gly-OH	92	190–192	9.86	9.94	284	273
CCpO-DL-Pro-DL-Phe-Gly-OH	99[c]	105–143	9.74	9.60	431	411
CCpO-DL-Met-Gly-OH	93	150–153	8.80	8.81	318	320
CCpO-L-Ileu-Gly-OH	78	135–138	9.33	9.21	300	308
CCpO-DL-Norval-Gly-OH	72	125–128	9.79	9.90	286	291
CCpO-L-Val-L-Ser-OH	68	183–186	8.85	8.78		
CCpO-Gly-β-Ala-L-Leu-D-Thr-OH	44	158–164	11.85	11.75	472	482
CChO-DL-Phe-Gly-OH[a]	92	160–161	8.04	7.98	348	351
CChO-Gly-L-Leu-Gly-OH	97	191–193	11.31	11.29	371	387
C-*p*-MoBzO-Gly-DL-Met-OH[d]	62	137–139	7.57	7.67	370	362
C-*p*-MoBzO-DL-Met-Gly-OH	61	122–125	7.57	7.73		
C-di-PrO-Gly-DL-Phe-OH[e]	37	134–139	7.68	7.80	364	363

[a] See Table III for abbreviations. [b] Value for crude product. [c] Mixture of racemates obtained as a sirup. Upon trituration with ether 31% crystallized. [d] C-*p*-MoBzO represents carbo-*p*-methoxybenzoxy. [e] C-di-Pro represents carbo-(diisopropyl)-methoxy.

Anal. Calcd. for $C_{22}H_{36}N_6O_5 \cdot 2HCl$: N, 15.64; Cl, 13.19. Found: N, 16.07; Cl, 12.99.

Peptide Ester Hydrobromides.—The following examples illustrate the removal of a carbocyclopentyloxy group to give a peptide ester. To 19.9 g. of carbocyclopentyloxyglycylglycine methyl ester was added 67 ml. of acetic acid saturated with hydrogen bromide. The suspension was warmed on the steam-bath to effect solution. After 25 minutes, scratching induced crystallization. Ether was added to complete the precipitation of the glycylglycine methyl ester hydrobromide. The yield of product melting at 165–172° amounted to 83%.

Anal. Calcd. for $C_5H_{10}N_2O_3 \cdot HBr$: N, 12.34. Found: N, 12.46.

In like fashion was prepared glycyl-L-leucine methyl ester hydrobromide in 62% yield, m.p. 151–153°.

Anal. Calcd. for $C_9H_{18}N_2O_3 \cdot HBr$: Br, 28.22. Found: Br, 28.38.

Isobutyl 4-Methylphenyl Carbonate.—Fifty-four grams of *p*-cresol was dissolved in a solution of 20 g. of sodium hydroxide in 100 ml. of water. This solution was dripped into a chilled solution of 68 g. (66 ml.) of isobutyl chloroformate in 100 ml. of chloroform over a period of a half-hour. The mixture was stirred for one hour after the addition was complete, after which the chloroform layer was separated, dried over Drierite and concentrated to a yellow liquid. The product was distilled at 135–142° at 13 to 15 mm.; 81.6 g. (79% yield) of product was obtained.

Anal. Calcd. for $C_{12}H_{16}O_3$: C, 69.18; H, 7.74. Found: C, 69.56; H, 7.80.

Isopropyl phenyl carbonate was prepared in 99% yield by the same method.

***t*-Butyl 4-Methylphenyl Carbonate.**—A suspension of 0.2 mole of sodium *t*-butoxide in 200 ml. of toluene was dripped into a chilled solution of 34.1 g. of 4-methylphenyl chloroformate in 175 ml. of benzene over a period of 45 minutes. The mixture was stirred at room temperature for 1.5 hours and allowed to stand overnight. The mixture was filtered, washed with ice-cold water, dried over Drierite and concentrated to give a mixture of oil and crystalline material. The crystals, which proved to be di-4-methylphenyl carbonate, were filtered off and the oil thoroughly chilled. Scratching of the oil gave 21.0 g. of crystals (50.5% yield) which melted at 48–52°. The product was used without further purification.

Anal. Calcd. for $C_{12}H_{16}O_3$: C, 69.18; H, 7.74. Found: C, 68.16; H, 8.60.

Urethans of Amino Acids *via* Mixed Carbonates.—Amino acid esters were refluxed in methyl alcohol with the appropriate mixed carbonates for 3 to 4 hours, followed by saponification using 2 equivalents of sodium hydroxide. After acidification, the oily products were taken up in ether and extracted with sodium bicarbonate solution. Acidification gave the carboalkoxyamino acid which was extracted from aqueous solution with ether. The yields of carbisobutoxy- and carbisopropoxyglycine were 30 and 20%, respectively. Carbo-*t*-butoxyglycine and carbo-*t*-butoxy-DL-alanine were obtained in only trace yields.

3-Benzylpiperazine-2,5-dione.—Three grams of carbo-*t*-butoxyglycyl-DL-phenylalanylglycine was refluxed in 25 ml. of glacial acetic acid for 4 hours. Carbon dioxide evolution, which began at the boiling point, had apparently ceased after this time. Acetic acid was removed *in vacuo*, leaving a brown, pasty residue. Trituration with methanol gave 0.6 g. of crystalline product which melted at 276–278° dec.

Anal. Calcd. for $C_{11}H_{12}N_2O_2$: N(K), 13.47; N(AP), 0. Found: N(K), 13.24; N(AP), 0.

The same compound was prepared by refluxing 2.1 g. of phenylalanylglycylglycine in acetic acid. After 5 hours 1.0 g. of product melting at 277–279° dec. was isolated. Found: N(K), 13.73.

Acknowledgment.—The authors wish to thank Mrs. Mary Graham for technical assistance and Mr. M. E. Auerbach and Mr. K. D. Fleischer and staff for analytical results.

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