Anal. Calcd. for $C_{11}H_{16}N_5O_3 \cdot HI \cdot H_2O$: C, 32.2; H, 4.5; N, 17.1. Found: C, 32.2; H, 4.4; N, 17.3.

Hydrolysis of 2′-Deoxy-1-methyladenosine (Water).—A dilute solution of 2′-deoxy-1-methyladenosine hydroiodide (20 mg. in 0.5 ml. of water) was heated for 20 min. on a steam-bath. The solution was then cooled to room temperature and adjusted to *p*H 7.5 with concentrated aqueous ammonia. This solution was subjected to paper chromatography and gave evidence of the presence of 1-methyladenine and 2-deoxy-D-ribose.

1-Methyladenine.[24]—2′-Deoxy-1-methyladenosine hydroiodide (IV, 10 g.), in 100 ml. of methanol, was heated on a steam-bath for 30 min. The mixture was allowed to cool at room temperature for 10 hr. The precipitate that separated was filtered and dissolved in 20 ml. of water, and the resulting solution was treated with Celite and filtered. The filtrate was adjusted to *p*H 8.5 with concentrated aqueous ammonia. The white precipitate that separated was filtered, washed with cold water, and dried under a heat ray lamp to yield 2.5 g. of crude product. For analysis a small sample was recrystallized twice from water. The purified product softened at 290–295° and melted with decomposition at 296–299°; ultraviolet data: *p*H 1, λ_{max} 257.5 mμ, ε 11,800; λ_{min} 227 mμ, ε 1,400; *p*H 11, λ_{max} 268.5 mμ, ε 12,100; λ_{min} 241.5 mμ, ε 1,400.

Anal. Calcd. for $C_7H_7N_5$: C, 48.3; H, 4.7; N, 47.0. Found: C, 48.5; H, 5.1; N, 46.5.

2′-Deoxy-N⁶-methyladenosine (2′-Deoxy-6-methylamino-9-β-D-ribofuranosylpurine).—The iodide salt of 2′-deoxy-1-methyladenosine monohydrate (IV, 2 g.), in 50 ml. of 0.2 *N* sodium hydroxide, was heated on a steam-bath for 30 min. and then chilled to 28°. The resulting solution was carefully neutralized to *p*H 7 with a 10% aqueous solution of *p*-toluenesulfonic acid. The mixture was evaporated to dryness under reduced pressure on a rotary evaporator, and the residue was extracted with 50 ml. of cold methanol in 2 portions. The insoluble residue was recrystallized from 35 ml. of hot methanol to yield 0.6 g. (dried at 80° for 2 hr.), m.p. 206–208° (block preheated to 180°). This product chromatogrammed as a single homogeneous spot in three different solvents identical to the previous reported R_f values for the same compound prepared by enzymatic means.[13]

Hydrolysis of 2′-Deoxy-N⁶-methyladenosine (IX).—A small sample of IX (0.2 g.) in 5 ml. of 0.1 *N* hydrochloric acid was heated for 10 min. on a steam-bath. The resulting solution was neutralized with concentrated aqueous ammonia and chromatogrammed in three different solvents. The chromatograms indicated a single ultraviolet absorbing spot identical to that of an authentic sample of 6-methylaminopurine.[26] A dark, homogeneous spot identical to that obtained from 2′-deoxyribose resulted after developing the chromatograms with silver nitrate in acetone followed by alcoholic potassium hydroxide. When the neutralized hydrolysate was allowed to stand at room temperature, a white precipitate separated. The product proved to be identical to 6-methylaminopurine as judged by ultraviolet and infrared spectra.

1-Methylinosine.—Inosine (5 g.) was added to 50 ml. of N,N-dimethylacetamide, containing 3 g. of potassium carbonate, heated at 80°. The mixture was treated with 3.5 g. of methyl *p*-toluenesulfonate (added dropwise) and the solution stirred at 100° for 2 hr. The resulting solution was treated with Celite and filtered and the filtrate diluted to 250 ml. with acetone. The precipitate that separated was collected, washed with 75 ml. of acetone, dissolved in 300 ml. of methanol, and neutralized to *p*H 7 with *p*-toluenesulfonic acid in methanol. The solution was allowed to stand at room temperature for 15 hr., and the crystalline potassium tosylate was filtered. The solution was then refrigerated at 15° for 48 hr. and filtered and the filtrate evaporated in a rotary evaporator at 80°. The residue was suspended in 75 ml. of isopropyl alcohol and dissolved by addition of methanol. The filtered solution was allowed to crystallize for 24 hr. at 15°, and the white product (2 g.) that separated was filtered and finally recrystallized from ethyl alcohol. The pure product melted at 210–212° (lit.[31] 211–212°).

Anal. Calcd. for $C_{11}H_{14}N_5O_4$: C, 46.8; H, 5.0; N, 19.9. Found: C, 46.9; H, 5.4; N, 19.6.

[CONTRIBUTION FROM THE ORGANIC CHEMICAL RESEARCH SECTION, LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID CO., PEARL RIVER, N. Y.]

The Tertiary Butyl Group as a Blocking Agent for Hydroxyl, Sulfhydryl and Amido Functions in Peptide Synthesis

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The *tert*-butyl group has been used to protect hydroxyl groups during peptide synthesis and conditions for its subsequent removal have been determined. Its removal from a sulfhydryl or amido group was too difficult for practical application to peptide synthesis. Several intermediates are described.

The reaction to form a peptide bond between the ester of an amino acid and an acylated amino acid is usually a straightforward procedure if the starting amino acids are the simple ones such as glycine, alanine or valine, which have no additional reactive centers. However, with amino acids bearing hydroxyl or sulfhydryl groups, *O*- or *S*-acylation is a common and an expected side reaction. Where a simple amido group is exposed, as in asparagine, many peptide-forming reagents lead to some nitrile formation[1,2] as well as other anomalies.[3] In addition to intermolecular side reactions, compounds containing glutamine or asparagine have a tendency to form undesirable cyclic structures.[4–6] To avoid such side reactions a number of groups have been used to protect amido, hydroxyl and sulfhydryl functions during peptide synthesis. A widely used means of O-protection has been acylation by the tosyl,[7–11] acetyl,[12–15] carbobenzoxy[16–18] and the *p*-nitrocarbobenzoxy groups.[19] Less used are the benzylsulfonyl,[20] β-naphthalenesulfonyl[21] and benzoyl derivatives.[22]

Cysteine has been dicarbobenzoxylated and conditions for removal of the two acyl groups were determined.[23]

Among the ether blocking groups for the hydroxyl function the benzyl[24–26] group is outstanding because of its easy removal by catalytic hydrogenation. The

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methyl ethers[27,28] have been used in peptide synthesis, but conditions for the removal of the methyl group, as given by Flěs and Balenović,[29] would seem too severe for application to peptides in general.

The benzyl group has been extremely valuable in peptide synthesis for almost two decades in forming a thioether of cysteine,[30] and more recently the *p*-nitrobenzyl group has been shown to be useful.[31] Izumiya[32] prepared the methyl and butyl thioethers of cysteine to study the Walden inversion, but use of such ethers as blocking groups for cysteine seems impractical. The very promising trityl group has been used to block the sulfhydryl function of cysteine by Amiard and his coworkers,[33] as well as by Zervas and Theodoropoulos.[34] The benzylthiomethyl group has been recently[35] introduced as a novel protecting group for the sulfhydryl function, but difficulties in removal have been encountered.[36]

Another interesting approach to the treatment of hydroxyl or sulfhydryl groups is that of Sheehan,[37] who used lactones or thiolactones of serine and β,β-dimethylcysteine as activated intermediates in peptide synthesis. However, this method simultaneously unblocks the "extra" function permitting it to enter into side reactions at later steps.

The use of *N,N'*-dicyclohexylcarbodiimide to form peptides with unblocked hydroxyamino acids has been described.[38] However, better results were obtained when the hydroxyl group was blocked.[25]

Protecting groups for the amino function in peptide synthesis are few in number. Frankel, Liwschitz and Zilkha[39] prepared N^{β}-benzyl-DL-asparagine, but were unable to remove the benzyl group. Recently the use of methylene-L-asparagine was demonstrated by Stammer.[40] An interesting use of the xanthyl protecting group for the amido function of glutamine was shown by Akabori and co-workers.[41]

The great value in peptide syntheses of the *tert*-butyl group has been demonstrated by use of intermediate *tert*-butyloxycarbonyl amino acids[42-44] and *tert*-butyl amino acid esters.[45-56] In the course of our preparation of *tert*-butyl esters of acylated amino acids it was discovered that those having an available hydroxyl group also simultaneously formed the corresponding *tert*-butyl ethers. The application of these ethers as valuable intermediates in a peptide synthesis was obvious because of the easy reversibility of the isobutylene addition step. When our study was almost completed, a short publication appeared[57] confirming our own work. The need for a good hydroxyl protecting group becomes apparent when one considers the incorporation of any one of the large number of naturally occurring hydroxy and dihydroxy amino acids[58] into a peptide.

Of amino acids which have an "extra" functional group, the following were selected as examples: serine to give the ether of a primary alcohol, threonine to give the ether of a secondary alcohol, hydroxyproline to yield the ether of an alicyclic alcohol, tyrosine to form the ether of a phenol and cysteine to yield a thioether. The preparation of *N*-acylated amino acid ether-esters is illustrated in examples I and II. The properties of the *N*-acylated amino acid ether-esters which were prepared are found in Table I. The nitrogen-blocking group was removed from each of the ether-ester derivatives as well as from some related compounds to yield the free bases as illustrated in example III. The properties of the free bases are listed in Table II.

In our synthesis of *tert*-butyl *N*-carbobenzoxy-*O*-*tert*-butyl-L-threoninate (more easily written Z·thr-(O-*t*-Bu)·O-*t*-Bu(L)[59] with the protected hydroxyl or sulfhydryl group enclosed in parentheses) as in the case of tyrosine analog II, the formation of the ether by isobutylene addition did not go to completion. The carbobenzoxylated reaction products were separated by passage through an alumina column, which retained Z·thr·O-*t*-Bu(L). More simply, the crude reaction products were reduced over 10% palladium-on-charcoal and the mixture of H·thr(O-*t*-Bu)·O-*t*-Bu(L) and H·thr·O-*t*-Bu(L) was separated by crystallization of the phosphite salts (ex. IV). The two bases could not be separated by fractional distillation.

The phthaloyl protecting group was removed from the crude Phth·cy(S-*t*-Bu)·O-*t*-Bu(L) using hydrazine.[49,60] The free bases of the *tert*-butyl esters of the *tert*-butyloxy amino acids and the *tert*-butylmercapto amino acid were all easily distillable liquids and entered into peptide-forming reactions (ex. V to VII). The derivatives with an ether group melted lower than those with a hydroxyl group free (ex. VIII, IX).

Two hydrazides, Z·ser(O-*t*-Bu)·$NHNH_2$(L) and Z·ser(O-*t*-Bu)·$NHNH_2$(DL), were prepared as intermediates for peptide synthesis (ex. X).

O-Acetyl-*N*-carbobenzoxy-DL-serine was prepared (ex. XI) in a different manner from that described,[14] since it was felt that there was a danger of some loss of the carbobenzoxy group and difficulty in preparing *O*-acetyl-*N*-carbobenzoxyserine had been reported.[61] This was converted to the *tert*-butyl ester (ex. XII). Subsequent removal of the acetyl group with sodium hydroxide solution caused serious side reactions to occur. The major product may have been Z·gly·O-*t*-Bu;

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TABLE I

PREPARATION OF ESTER–*tert*-BUTYL ETHERS BY ISOBUTYLENE ADDITION IN METHYLENE CHLORIDE IN PRESENCE OF H_2SO_4–CATALYST

Product[a]	Conversion, %	$n^{20}D$	Analyses.[b] % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	$[\alpha]^{25}D$ (c, EtOH)
Phth·cy(S–*t*-Bu)·O–*t*-Bu(L)[68]	73	....	62.61	7.19	3.84	60.19	6.82	4.28	...
Z·hypro(O–*t*-Bu)·O–*t*-Bu(L)[69]	79	1.4949	66.82	8.28	3.71	66.72	8.20	3.71	−37.6° (2.56)
Z·ser(O–*t*-Bu)·O–*t*-Bu(DL)[70]	70	1.4858	64.93	8.32	3.99	65.74	7.88	4.16	...
Z·ser(O–*t*-Bu)·O–*t*-Bu(L)[70]	74	1.4816	64.93	8.32	3.99	64.20	8.10	4.11	− 1.6° (1.88)
Z·thr(O–*t*-Bu)·O–*t*-Bu(L)[71]	88[c]	....	65.73	8.55	3.83	64.62	8.31	4.14	...
Z·ser(O–*t*-Bu)·OMe(DL)[72]	72[d]	....	62.12	7.49	4.53	62.14	7.55	4.47	...
Z·ser(O–*t*-Bu)·OMe(L)[72]	60[e]	....	62.12	7.49	4.53	61.84	7.58	4.49	+ 6° (1.996)
Z·tyr(O–*t*-Bu)·O–*t*-Bu[f]									

[a] The lit. references are indicated for the source or means of preparing the starting materials. All compounds were prepared by the method illustrated in example I, Experimental. [b] Analysis of crude product unless otherwise indicated. [c] The analysis is for the material before treatment on the alumina column, ex. IV. [d] Crystalline solid, m.p. 78.5–79.0° (from petroleum ether, b.p. 90–100°). [e] Crystalline solid, m.p. 40–41° (from petroleum ether, b.p. 90–100°). [f] See ex. II in Experimental.

similar reactions have been reported.[58] Since removal of acyl groups from serine by dilute alkali has been reported[62] to be very rapid, it is possible that a shorter reaction time might have prevented side reactions. However, when it was found that ammonia would remove the acyl group easily (ex. XIII), the reaction with sodium hydroxide solution was not investigated further; Z·ser·O–*t*-Bu (DL) was easily hydrogenated to the free base (Table II).

An attempt to prepare Z·ser·O–*t*-Bu (DL) from Z·ser·OAg(DL) and *tert*-butyl iodide by a known method[47] yielded a mixture of products.

Also prepared as potential intermediates for peptide synthesis, were Z·ser(O–*t*-Bu)·OH(L) and -(DL) (ex. XIV).

Conditions for removal of *tert*-butyl groups were studied on H·ser(O–*t*-Bu)·O–*t*-Bu(DL), H·cy(S–*t*-Bu)·O–*t*-Bu(L) and H·tyr(O–*t*-Bu)·O–*t*-Bu(L). Removal of the *tert*-butyl group from a thioether failed to go to completion in contrast to the oxyethers. Amiard and his co-workers[33] successfully removed the trityl group using hydrogen chloride in chloroform. However,

$$\begin{matrix} NO_2S\text{–}t\text{-Bu} \\ | \quad | \end{matrix}$$

with Z·arg–cy·O–*t*-Bu (2L), only the ester function lost isobutylene (ex. XV), under similar conditions. In contrast, removal of the *tert*-butyl groups from Phth·gly-ser(O–*t*-Bu)·O–*t*-Bu(L) was straightforward (ex. XVI) to yield Phth.gly-ser·OH(L).

To follow the removal of a *tert*-butyl group chromatographically H·ser·O–*t*-Bu(DL), H·ser(O–*t*-Bu)·OH(DL) (ex. XVII), and H·cy(S–*t*-Bu)·OH(ex. XVIII) were synthesized.

Since removal of the phthaloyl protecting group differs from the removal of the carbobenzoxy group, the preparation of H·*cy*(S–*t*-Bu)·O–*t*-Bu has been given (ex. XIX).

The best cleavage of the oxyethers was achieved with hydrogen bromide in glacial acetic acid and hydrogen chloride in chloroform as shown in Table III.

The chromatographic methods and results are described in the Experimental part under ex. A, B and C in Experimental, section 2.

A study was made to determine if a *tert*-butyl group could be used as an amide-protecting group. Although Ritter and Minieri[63] showed that acid hydrolysis of *tert*-butyl amides led to formation of isobutylene, ammonia and the corresponding acid, Lacey[64] found that *N*-*tert*-butylbenzamide on treatment with 98% sulfur acid for 5 min. at room temperature gave a 99% yield of benzamide. Two *N*-*tert*-butylamides, 1-tosyl-5-*N'*-*tert*-butylcarboxamido-2-pyrrolidone (ex. XX) and *N*-*tert*-butylcarbobenzoxy-DL-phenylalanine amide (ex. XXI), were prepared from *tert*-butylamine and the corresponding acid. The synthesis of several analogs was attempted using similar procedures (ex. XXII). A third *tert*-butyl amide was prepared (ex. XXIII) by the addition of isobutylene to carbobenzoxy-β-cyano-L-alanine[65] to yield two products, *tert*-butyl N^{β}-*tert*-butyl N^{α}-carbobenzoxy-L-asparaginate and *tert*-butyl carbobenzoxy-β-cyano-L-alaninate. Hydration of the latter compound yielded *tert*-butyl carbobenzoxy-L-asparaginate, m.p. 107–108.5°. Taschner and his co-workers[66] reported a melting point of 70° (which we assumed to be the L-compound). Attempts to duplicate his work under the described conditions failed. When the compound was prepared[47] using the silver salt of the acylated amino acid and *tert*-butyl iodide, the product melted at 105–106°. The procedure of Staab,[67] *et al.*, failed to yield *tert*-butyl carbobenzoxy-L-asparaginate.

Reduction of *tert*-butyl N^{β}-*tert*-butyl-N^{α}-carbobenzoxy-L-asparaginate in the presence of 10% palladium-on-charcoal proceeded well and the resulting free base was isolated with no difficulty. An attempted removal of the N^{β}-*tert*-butyl group from the carbobenzoxy derivative using hydrogen bromide in glacial acetic acid was unsuccessful.

Sulfuric acid treatment may have given partial reaction in the case of 1-tosyl-5-*N'*-*tert*-butylcarboxamido-2-pyrrolidone, but gave decomposition in the case of *tert*-butyl N^{β}-*tert*-butyl-L-asparaginate and of *N*-*tert*-butyl carbobenzoxy-DL-phenylalanine amide.

Experimental

Sec. 1. **Preparations.**[73] ***tert*-Butyl *O*-*tert*-Butyl-*N*-carbobenzoxy-DL-serinate** (I).—Carbobenzoxy-DL-serine[70] (12 g.) was suspended in 500 ml. of methylene chloride with stirring. Isobutylene was bubbled through the mixture and 2 ml. of concd. sulfuric acid was slowly added. Addition of isobutylene was continued for a period of 3 hr. The flask was then stoppered and permitted to stand overnight at room temperature. The solution was successively extracted with 200-ml. portions of 10% sodium bicarbonate, water, *N* hydrochloric acid, and water, then dried over anhydrous sodium sulfate. The solvent was removed by evaporation to give a residue of 12.2 g. (70%) as a

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TABLE II

tert-BUTYL DERIVATIVES OF AMINO ACIDS

Compound[a]	Yield, %	Analyses, % Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N	B.p. °C.	B.p. Mm.	$n^{20}D$	d^{20}_4	$[\alpha]^{25}D$ (c)
H·hypro(O-*t*-Bu)·O-*t*-Bu(L)	68	64.16	10.36	5.76	63.91	10.35	5.58	90	0.4	1.4460	0.9676	− 8.0° (100)
H·cy(S-*t*-Bu)·O-*t*-Bu(L)	38[b]	56.62	9.94	6.01	56.83	9.98	6.33	95–97	.4	1.4654	0.9794	+10.3° (100)
H·tyr(O-*t*-Bu)·O-*t*-Bu(L)	38	69.59	9.28	4.77	69.90	9.16	4.77	127	.25	1.4904	1.0096	+25.45° (100)
H·thr(O-*t*-Bu)·O-*t*-Bu(L)	35	62.30	10.85	6.06	62.15	11.16	5.87	70	.75	1.4322	0.9410	+ 3.7° (1.34 EtOH)
H·ser(O-*t*-Bu)·O-*t*-Bu(L)	80	60.80	10.67	6.45	61.08	10.74	6.43	47	.25	1.4285	.9250	− 3.7°(1.955, EtOH)
H·ser(O-*t*-Bu)·O-*t*-Bu(DL)	55	60.80	10.67	6.45	60.90	10.69	6.76	73	1.5	1.4250	.9354	...
H·ser·O-*t*-Bu(DL)	64	52.15	9.58	8.69	52.05	9.40	8.72	50–51		....	....	...
H·ser(O-*t*-Bu)·OH.$^1/_4$H$_2$O(DL)	60	50.75	9.37	8.46	50.39	9.34	8.49	200–205[c]		....	....	...
H·ser(O-*t*-Bu)·OH(L) NH-*t*-Bu	50	52.15	9.38	8.69	52.24	9.69	8.49	203–204[c]		....	....	−13.2° (0.91, H$_2$O)
H·asp·O-*t*-Bu(L)	75	58.99	9.90	11.47	58.63	9.94	11.57	123	0.4	1.4627	....	+1.5° (2.8, EtOH)
H·asp(NH$_2$)·O-*t*-Bu(L)	73	51.05	8.57	14.88	51.34	8.42	14.91	101–102[c]		....	.. .	+0.24° (2.01, EtOH)

[a] Prepared by hydrogenation of the carbobenzoxy compound, except the cysteine derivative, which was prepared by hydrazinolyses of the corresponding phthaloyl intermediate. [b] The yield includes the material isolated as the hydrochloride salt XX. [c] M.p., °C.

cloudy oil. The oil was filtered through Celite[74] diatomaceous earth and used in the next step without further purification (see Table I).

tert-Butyl *O-tert*-Butyl-L-tyrosinate (II).—Carbobenzoxy-L-tyrosine[75] (103 g., 0.327 mole) was added to 700 ml. of methylene chloride (solvent) which contained 3.0 ml. of concd. sulfuric acid as a catalyst. Gaseous isobutylene was bubbled into the stirred suspension with solution rapidly taking place. The solution was kept in a stoppered flask overnight. After extraction of the methylene chloride solution with 200 ml. of 5% sodium bicarbonate solution and subsequent evaporation of the methylene chloride and the excess isobutylene, a mixture of Z·tyr·O-*t*-Bu(L) and Z·tyr·(O-*t*-Bu)·O-*t*-Bu(L) amounting to 129 g. was obtained.

The mixture was reduced in 300 ml. of ethanol in the presence of 7.0 g. of 10% palladium-on-charcoal.[76] After removal of the catalyst by filtration, the filtrate was concentrated to a volume of 40 ml. at reduced pressure. The alcoholic solution was shaken with a mixture of *N* hydrochloric acid and methylene chloride. The aqueous layer was made alkaline with solid sodium bicarbonate, causing H·tyr·O-*t*-Bu to precipitate. On recrystallization from methanol–diisopropyl ether, 13.9 g. of product was obtained, m.p. 142–144°; lit. gives 144–145°,[45] 143–144°.[47] Evaporation of the methylene chloride solution left 60 g. of H·tyr-(O-*t*-Bu)·O-*t*-Bu(L) as the hydrochloride. One gram was recrystallized from isopropyl acetate giving a material with m.p. 159–160°. A second recrystallization left the melting point unchanged; $[\alpha]^{25}D$ +41° (*c* 3.14, ethanol). lit.[57] gives m.p. 154–155° and $[\alpha]^{25}D$ +42° (*c* 1.75, dimethylformamide).

Anal. Calcd. for $C_{17}H_{27}NO_3$.HCl: C, 61.89; H, 8.55; N, 4.24. Found: C, 61.93; H, 8.67; N, 4.25.

tert-Butyl *O-tert*-Butyl-DL-serinate (III).—*tert*-Butyl-*O-tert*-butyl-*N*-carbobenzoxy-DL-serinate (8.2 g., 0.028 mole) from I was dissolved in 100 ml. ethanol and 3.0 g. of palladium-on-charcoal as catalyst was added under a nitrogen atmosphere. The solution was hydrogenated for 3 hours until evolution of carbon dioxide had ceased. The catalyst was filtered under a nitrogen atmosphere and the product isolated by distillation of the filtrate at reduced pressure (see Table II).

tert-Butyl L-Threoninate and *tert*-Butyl *O-tert*-Butyl-L-threoninate (IV). Procedure A.—*tert*-Butyl *O-tert*-butyl carbobenzoxy-L-threoninate was prepared by the addition of isobutylene to carbobenzoxy-L-threonine[71] in the presence of sulfuric acid catalyst dissolved in methylene chloride as in I. Without further purification, this crude product was hydrogenated in ethanol over 10% palladium-on-charcoal at atmospheric pressure to yield a mixture of H·thr·O-*t*-Bu(L) and H·thr(O-*t*-Bu)·O-*t*-Bu-(L). The two bases could not be separated by fractional distillation. After their simultaneous conversion to the phosphite salts, the two salts were separated in the molar ratio of 2:1 of ether–ester to ester by a fractional crystallization from a mixture of ether–petroleum ether (b.p. 30–60°). The H·thr·O-*t*-Bu(L) salt crystallized first, m.p. 139–141°. Recrystallization from ethanol–petroleum ether raised the melting point to 140–141°. The H·thr(O-*t*-Bu)·O-*t*-Bu(L) salt, m.p. 70–72°, crystallized after the addition of petroleum ether to the filtrate. Recrystallization from ether–petroleum raised the melting point to 74–76°. Chromatography in BAM (defined in section 2) on silica gel plates gave R_f values of 0.65 and 0.75 for mono- and di-*tert*-butyl derivatives, respectively.

Anal. Calcd. for $C_8H_{17}NO_3 \cdot H_3PO_3$: C, 37.38; H, 7.83; N, 5.45; P, 12.05. Found: C, 37.67; H, 7.82; N, 5.59; P, 11.93; $[\alpha]^{25}D$ −12.9° (*c* 1.341, water). Calcd. for $C_{12}H_{25}NO_3 \cdot H_3PO_3 \cdot 1/2\ H_2O$: C, 44.70; H, 9.06; N, 4.35; P, 9.62. Found: C, 44.40; H, 9.28; N, 4.26; P, 9.20; $[\alpha]^{25}D$ −20.0° (*c* 1.444, water).

Procedure B.—*tert*-Butyl-*O-tert*-butyl carbobenzoxy L-threoninate was purified on an alumina column to give a clear oil. A column with an inside diameter of 1.0 cm. was packed with neutral alumina to a height of 15 cm. A 330-mg. quantity of the products resulting from the reaction of Z·thr·OH(L) with isobutylene, as in I, was dissolved in 5 ml. of carbon tetrachloride. This solution was added on the column. The material was removed by gradient elution proceeding from chloroform to ethyl acetate to methanol. Thirty fractions each of a 10-ml. volume were collected.

The eight center fractions were found to contain only one material by a determination employing thin-layer chromatograms (see Experimental, section 2) using the solvent system chloroform–methanol 2:1. The spots, developed by tolidine after chlorine treatment, all had an R_f value of 0.77. The starting material contained two materials with R_f values of 0.30 and 0.76. The material with an R_f value of 0.30 remained on the column. The amount of Z·thr(O-*t*-Bu)·O-*t*-Bu(L) in the eight center fractions was 100 mg.

Anal. Calcd. for $C_{20}H_{30}NO_5$: C, 65.73; H, 8.55; N, 3.83. Found: C, 65.39; H, 8.63; N, 3.90.

tert-Butyl *O-tert*-butyl-L-threoninate was then prepared by hydrogenation of the carbobenzoxy derivative as in III.

tert-Butyl Carbobenzoxy-L-nitroarginyl-S-*tert*-butyl-L-cysteinate (V).—*tert*-Butyl *S-tert*-butylcysteinate (L) (2.19 g., 0.0094 mole) and carbobenzoxy-L-nitroarginine[77] (3.53 g., 0.01 mole) were added to 25 ml. of tetrahydrofuran (distilled from calcium hydride). Then dicyclohexylcarbodiimide[78,79] (2.27 g., (0.011 mole) was added and a temperature of 15–20° was maintained for 1 hour. Precipitation of dicyclohexylurea was immediate. The reaction mixture was left standing at the ambient temperature for an additional 20 hr. Then 1 ml. of acetic acid was added, and after a few minutes the urea was separated and the filtrate was evaporated to dryness *via* a water aspirator. The residue was dissolved in 50 ml. of methylene chloride and additional urea was removed by filtration. The methylene chloride layer was washed with 50 ml. of *N* hydrochloric acid and 50 ml. of 5% sodium bicarbonate. The methylene chloride layer was dried over anhydrous sodium sulfate and evaporated to dryness in an open dish to give a quantitative yield of crude product, m.p. 103–125°. Successive recrystallizations from aqueous ethanol, ethyl acetate–petroleum ether, and toluene gave a 60% yield, m.p. 140–141°, $[\alpha]^{25}D$ −17° (*c* 2.1, ethanol).

Anal. Calcd. for $C_{25}H_{40}N_6O_7S$: C, 52.80; H, 7.09; N, 14.78; S, 5.64. Found: C, 52.67; H, 7.21; N, 15.10; S, 6.13.

tert-Butyl Carbobenzoxy-L-phenylalanyl-*O-tert*-butyl-L-tyrosinate (VI).—Z·phe·OH(L)[80,81] (2.99 g., 0.010 mole) and H·tyr-(O-*t*-Bu)·O-*t*-Bu(L) II (2.93 g., 0.010 mole) were added to 7 ml. of dimethoxyethane (solvent). Tetraethyl pyrophosphite[82] (3.0 ml., 0.011 mole) was added and the solution caused to reflux for a period of 30 minutes. Isolation of the product was accomplished by adding the solution to 75 ml. of ice–water mixture. The precipitate formed a gum from which the supernatant solution was decanted. The residue was triturated with 20 ml. of a 5% sodium bicarbonate solution and then with 20 ml. of *N* hydrochloric acid. The material was washed with 20 ml. of

(74) Celite is the trade mark of Johns-Manville for diatomaceous silica products.

(75) Supplied by Mann Research Lab., Inc., N. Y.

(76) Supplied by Baker and Co., Inc. Catalysts.

(77) Supplied by Cyclo Chemical Corp., Los Angeles, Calif.

(78) J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, **77**, 1067 (1955).

(79) H. G. Khorana, *Chemistry & Industry*, 1087 (1955).

(80) W. Grassmann and E. Wunsch, *Ber.*, **91**, 465 (1958).

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

(82) G. W. Anderson, J. Blodinger and A. D. Welcher, *J. Am. Chem. Soc.*, **74**, 5309 (1952).

water after each operation. The gum slowly crystallized yielding 5.45 g. (93%) of product, m.p. 59–65°. Recrystallization from ether–petroleum ether produced 2.72 g. (47%), m.p. 70.0–70.5°, $[\alpha]^{25}D$ −8.0° (*c* 4.2, ethanol).

Anal. Calcd. for $C_{34}H_{42}N_2O_6 \cdot 1/2\ H_2O$: C, 70.25; H, 7.54; N, 4.90. Found: C, 69.96; H, 7.43; N, 4.80.

tert-Butyl **Phthaloylglycyl-*O*-*tert*-butyl-DL-serinate (VII).**—Phthaloylglycine[83] (1.03 g., 0.005 mole), *tert*-butyl *o*-*tert*-butyl-DL-serinate (III) (1.08 g., 0.005 mole) and tetraethyl pyrophosphite (1.5 ml., 0.0055 mole) were combined in 10 ml. of dimethoxyethane (solvent) under anhydrous conditions and the solution heated on a steam-bath for 30 min. After cooling slightly, the reaction mixture was added to 30 ml. of ice-water causing an oily precipitate to form, which crystallized on storage at 0°. The crystals were collected by filtration and the residue washed with ice-water, then successively washed with 25-ml. portions of 10% sodium bicarbonate, water, *N* hydrochloric acid, and water. After drying, the melting point was 134–135°. The product was, recrystallized twice from ethanol–water to give 0.8 g. (40%), m.p. 136.5–137°.

Anal. Calcd. for $C_{21}H_{28}N_2O_6 \cdot 0.25\ H_2O$: C, 61.65; H, 7.03; N, 6.85; K.F., 1.1. Found: C, 61.68; H, 6.18; N, 7.07; K.F., 1.37.

tert-Butyl **phthaloylglycyl-*O*-*tert*-butyl-L-serinate** was prepared in a similar fashion to the DL-analog. The yield was 68%, m.p. 88–89°, $[\alpha]^{25}D$ +6.7° (*c* 2.38, ethanol).

Anal. Calcd. for $C_{21}H_{28}N_2O_6$: C, 62.36; H, 6.98; N, 6.93. Found: C, 62.45; H, 7.10; N, 6.86.

tert-Butyl **Carbobenzoxy-L-prolyl-L-tyrosinate (VIII).**—H·tyr·O-*t*-Bu(L)[45,47] (2.37 g., 0.010 mole) and L-Z·pro·O-*p*-$C_6H_4NO_2$[84] (3.70 g., 0.010 mole) were combined in 50 ml. of methylene chloride (solvent). Very gentle warming was applied to effect complete solution, which occurred within a period of 5 minutes. The solution was kept in a stoppered flask for 60 hr. at ambient temperature. The product was isolated by extraction of the solution with 3 × 20 ml. of 5% ammonium hydroxide, then water (2 × 10 ml.). The solution was next extracted with 10 ml. of *N* hydrochloric acid and finally washed with 10 ml. of water. After drying over anhydrous sodium sulfate and subsequent evaporation of the methylene chloride, 4.67 g. of crude residue remained. The product slowly crystallized and melted at 113–116°. An extraction with a small amount of diisopropyl ether raised the m.p. to 121–123°. A recrystallization from a methanol–water mixture raised the m.p. to 122–123° with 3.30 g. (71%) remaining $[\alpha]^{25}D$ −31.6° (*c* 2.06, ethanol).

Anal. Calcd. for $C_{26}H_{32}N_2O_6$: C, 66.65; H, 6.88; N, 5.98. Found: C, 66.63; H, 6.98; N, 6.09.

tert-Butyl **Carbobenzoxy-L-prolyl-*O*-*tert*-butyl-L-tyrosinate (IX).**—The ester[84] Z·pro·O-*p*-$C_6H_4NO_2$(L) (3.70 g., 0.010 mole) and the base H·tyr(O-*t*-Bu)·O-*t*-Bu(L) (II) (2.93 g., 0.010 mole) were combined in 30 ml. of methylene chloride. After remaining for a period of 40 hr. at room temperature, the solution was extracted with 25 ml. of *N* hydrochloric acid and 25 ml. of *N* sodium hydroxide. The solution was washed with 25 ml. of water after each operation. After drying the solution over anhydrous sodium sulfate, the methylene chloride was removed from the filtered mixture by evaporation of the filtrate in an open dish. The product slowly crystallized, giving 4.5 g. (86%) of material, m.p. *ca.* 100°. Two successive recrystallizations from diisopropyl ether raised the m.p. to 108.5–109.5°. The net yield was 2.61 g. (50%), $[\alpha]^{25}D$ −29.2° (*c* 1.96, ethanol).

Anal. Calcd. for $C_{30}H_{40}N_2O_6$: C, 68.68; H, 7.69; N, 5.34. Found: C, 68.59; H, 7.77; N, 5.54.

O-tert-**Butylcarbobenzoxy-DL-serine Hydrazide and *O-tert*-Butylcarbobenzoxy-L-serine Hydrazide (X).**—Methyl *O-tert*-butyl carbobenzoxy-DL-serinate (3.09 g., 0.01 mole), which was prepared as I with its corresponding properties listed in Table I, and hydrazine hydrate (0.485 ml., 0.011 mole) were dissolved in 35 ml. of methanol and the reaction mixture was permitted to stand at room temperature overnight. After a reflux period of 2.5 hr. the solvent was removed in vacuo and the solid residue triturated with 40 ml. of *N* hydrochloric acid. Filtration yielded 2.06 g. of crystalline material, m.p. 77–79°. The filtrate was made alkaline with solid sodium bicarbonate and extracted with 2 × 30 ml. of ether. Evaporation of the ether layer resulted in 0.68 g. (18%) of white crystals, m.p. 112–114°.

The recovered starting material was combined with hydrazine hydrate (99%) dissolved in ethanol and refluxed for 3 hr. Another 0.47 g. (m.p. 112–113°) of product was isolated in the same manner as before. The total, 1.05 g. (28%), was recrystallized from isopropyl ether yielding 0.63 g., m.p. 112–113°; then from ethyl acetate–petroleum ether (b.p. 30–60°) giving 0.5 g., m.p. 113.5–114°. The sample was dried in low vacuum at the boiling point of acetone.

(83) J. Billman and W. Harting, *J. Am. Chem. Soc.*, **70**, 1473 (1948).

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

Anal. Calcd. for $C_{15}H_{23}N_3O_4$: C, 58.23; H, 7.49; N, 13.58. Found: C, 58.39; H, 7.59; N, 13.50.

The L-analog was prepared in a similar manner in 13% yield, m.p. 112.5–113.5° (ethyl acetate–petroleum ether), $[\alpha]^{25}D$ +14.5° (*c* 1.98, ethanol).

Anal. Calcd. for $C_{15}H_{23}N_3O_4$: C, 58.23; H, 7.49; N, 13.58. Found: C, 58.23; H, 7.60; N, 13.67.

O-**Acetyl-*N*-carbobenzoxy-DL-serine (XI).**—Z·ser·OH[70](DL) (23.9 g., 0.10 mole) was dissolved in 40 ml. of pyridine and acetyl chloride (7.8 ml., 0.11 mole) was added dropwise to the stirred solution. Cooling was provided by an ice-bath. After a period of 2 hr. the material was added to 500 ml. of cold water and the solution extracted with ether. Acidification of the aqueous solution with concd. sulfuric acid caused some gummy material to precipitate. The supernatant liquid was quickly decanted and 5.5 g. (19%) of material slowly crystallized; m.p. 123°, lit.[14] 116–118°; *neut. equiv.* calcd. 281, found 277 and 282.

Anal. Calcd. for $C_{13}H_{15}NO_6$: C, 55.51; H, 5.38; N, 4.98. Found: C, 55.68; H, 5.59; N, 4.92.

The above experiment was repeated using 28 ml. of triethylamine (0.20 mole) and 500 ml. of methylene chloride as a solvent. This increased the yield to 26.7% and the m.p. was 121°. By extraction of the triethylamine at the end of the acylation reaction, the yield remained the same, but the melting point now was 123°.

tert-Butyl *O*-**Acetylcarbobenzoxy-DL-serinate (XII).**—Z·ser(OAc)·OH(DL) (XI), (4.20 g., 0.015 mole) was dissolved in 250 ml. of methylene chloride. Then concd. sulfuric acid was added (1.0 ml. as catalyst) and isobutylene passed into the solution until it was saturated. The flask was sealed and kept at room temperature for a period of 64 hr. The acidic materials were extracted with 100 ml. of 5% sodium bicarbonate. Removal of the methylene chloride, under vacuum, gave a quantitative yield of the ester, $n^{20}D$ 1.4909.

Anal. Calcd. for $C_{17}H_{23}NO_6 \cdot 0.5 \cdot H_2O$: C, 58.95; H, 6.98; N, 4.05. Found: C, 59.35; H, 7.35; N, 3.98.

tert-Butyl **Carbobenzoxy-DL-serinate (XIII).**—Ammonia gas (1.0 g., 0.59 mole) was dissolved in 50 ml. of methanol. *tert*-Butyl *O*-acetylcarbobenzoxy-DL-serinate hemihydrate (XII) (16.0 g., 0.0463 mole) was next added. The flask was sealed and left at the ambient temperature for a period of 16 hours. The excess solvent was removed *via* the water aspirator and the residue shaken with a 1:1 mixture of ether and water. The ether layer was separated and dried over anhydrous sodium sulfate. Evaporation of the ether yielded 12.3 g. (90.5%), the crystalline product melting at 76–77°. A recrystallization from ether–petroleum ether did not raise the melting point.

Anal. Calcd. for $C_{15}H_{21}NO_5$: C, 61.00; H, 7.17; N, 4.74. Found: C, 61.03; H, 7.09; N, 4.82.

An attempt to prepare this compound[47] from Z·ser·OAg and *tert*-butyl iodide gave a low yield of what apparently was a mixture of the ester and ether-ester.

O-tert-**Butylcarbobenzoxy-DL-serine (XIV).**—To a methanolic solution of Z·ser(O-*t*-Bu)·OMe(DL) (3.1 g.; 0.010 mole), similarly prepared as in I (properties listed in Table I), was added 10 ml. of 2 *N* sodium hydroxide solution. The solution was shaken manually for 10 min. and permitted to stand for an additional 15-min. period. The product was precipitated by the addition of acetic acid and then the methanol removed by distillation. The residue was extracted into methylene chloride and the solvent removed by evaporation. The latter residue was redissolved in 10% sodium bicarbonate solution, the solution extracted with ether, and the aqueous layer was acidified to yield 2.1 g. (71%) of crude product, m.p. 46–48°. Recrystallization from isopropyl alcohol–water raised the melting point to 48–50° with a recovery of 1.8 g. (61%).

Exposed to air, the material spontaneously formed a gum and recrystallized in a 3-day period. The material now melted at 58–60°. The chemical analyses for the two forms were the same.

Anal. Calcd. for $C_{11}H_{21}NO_5$: C, 61.00; H, 7.17; N, 4.74. Found: C, 60.98; H, 7.30; N, 4.59.

The optically active L-compound was similarly prepared, m.p. 87.0–87.5° (cyclohexane), $[\alpha]^{24}D$ +22.7° (*c* 1.986, ethanol).

Anal. Found: C, 61.07; H, 7.30; N, 4.78.

Carbobenzoxy Nitro-L-arginyl-*S*-*tert* butyl-L-cysteine (XV).—

$$\begin{array}{l} NO_2\ S\text{–}t\text{–}Bu \\ |\quad\ | \end{array}$$

Z·arg–cy·O-*t*-Bu(2L) (ex. V) (1.14 g., 0.0020 mole) was dissolved in 15 ml. of chloroform. Hydrogen chloride was bubbled into the solution for a period of 1 hour, during which time a precipitate appeared. After an additional 1-hour period, 20 ml. of chloroform was added to facilitate handling. The material was shaken with enough 5% sodium bicarbonate solution to neutralize all acids present. Acidification of the bicarbonate solution yielded 890 mg. of material, m.p. *ca.* 100°. Recrystal-

lization from aqueous methanol gave 800 mg. (77%), m.p. 99–101°, $[\alpha]^{25}D$ −8.3°(*c* 2.31, ethanol).

Anal. Calcd. for $C_{21}H_{32}N_6O_7S \cdot 1/2\ H_2O$: C, 48.36; H, 6.37; N, 16.11; S, 6.15. Found: C, 48.34; H, 6.55; N, 16.23; S, 6.27.

Phthaloylglycyl-L-serine (XVI).—*tert*-Butyl phthaloylglycyl-*O*-*tert*-butyl-L-serinate (1.01 g., (0.00250 mole) (VII) was dissolved in 15 ml. of chloroform and hydrogen bromide bubbled through the material for a 15-min. period. The reaction product was extracted with 20 ml. of a 5% sodium bicarbonate solution. The Phth·gly-ser·OH(L) was recovered by acidification of the aqueous layer with concd. hydrochloric acid. The crude yield was 670 mg., m.p. 198–199°. Two recrystallizations from 12 ml. of water raised the m.p. to 210° with a net yield of 402 mg. (53%), $[\alpha]^{25}D$ +5.2° (*c* 0.95, ethanol).

Anal. Calcd. for $C_{13}H_{12}N_2O_6 \cdot 0.5\ H_2O$. C, 51.83; H, 4.35; N, 9.30. Found: C, 51.81; H, 4.80; N, 8.92.

***O*-*tert*-Butyl-DL-serine and *O*-*tert*-Butyl-L-serine (XVII).**—*O*-*tert*-Butylcarbobenzoxy-DL-serine (2.95 g., 0.01 mole) (XIV) was dissolved in 100 ml. of ethanol. Then 2 g. of 10% palladium-on-charcoal was added under a nitrogen atmosphere and hydrogen was then bubbled through the suspension. After a 1-hour period the catalyst was filtered off under a nitrogen atmosphere. The ethanol was removed *in vacuo* to yield an off-white crystalline solid, m.p. 150–180° (decomposition). The product was recrystallized by dissolving it in 20 ml. of water and adding acetone until crystallization began. The yield was 0.97 g. (60%), m.p. 200–205° dec. After one more recrystallization from a water–acetone mixture no change in melting point was observed.

The L-analog was similarly prepared. The properties of both compounds are listed in Table II.

***S*-*tert*-Butyl-L-cysteine (XVIII).**—*tert*-Butyl *S*-*tert*-butyl-L-cysteinate hydrochloride (0.54 g., 0.002 mole) (XIX) was added to 4 ml. of saturated hydrogen bromide–acetic acid solution and the material allowed to stand at room temperature for a 5-minute period. The addition of 25 ml. of ether to the reaction mixture yielded white crystals, which were collected and washed with ether. The hydrobromide (0.4 g.) was dissolved in a small amount of water and the *p*H adjusted to 7 with ammonium hydroxide. The solution was evaporated to dryness and triturated with ethanol. Filtration gave 0.18 g., m.p. 203–206°. Recrystallization from water–acetone mixture changed the melting point to 203–204°, $[\alpha]^{25}D$ −27.2 ± 3.1° (*c* 1.691, water); R_f 0.20, *sec*-butyl alcohol–ammonium hydroxide (3%), 3:1 system on silica.

Anal. Calcd. for $C_7H_{15}NO_2S$: C, 47.44; H, 8.52; N, 7.90; S, 18.09. Found: C, 47.47; H, 8.38; N, 7.95; S, 18.21.

***tert*-Butyl *S*-*tert*-butyl-L-cysteinate (XIX).**—A 41-g. quantity of crude Phth·cy(S-*t*-Bu)·O-*t*-Bu(L) (0.11 mole) prepared as in I, was combined with hydrazine hydrate (6.25 g., 0.125 mole) in 150 ml. of ethanol. Phthalazine began precipitating immediately. After a period of 18 hours, the phthalazine was removed by filtration, and the solvent removed at reduced pressure. The residue was divided in half. One portion was distilled to yield the free base, 6.5 g. (0.0279 mole), the properties of which are given in Table II. From the chromatographic studies (ex. B, sect. 2) the free base appeared to have a trace of H·cySH·O-*t*-Bu(L).

The remaining portion of crude base was converted to the hydrochloride salt in cold ether. The crude yield of hydrochloride salt amounted to 8.59 g., m.p. *ca.* 165°. Recrystallization from a diisopropyl ether–ethanol mixture, 3:1, yielded the pure compound, 3.70 g. (0.0137 mole), m.p. 173° dec.; R_f in BAM (ex. B) 0.80 with a single ninhydrin positive spot. In BAW, only a single ninhydrin spot developed with the value 0.93.

Anal. Calcd. for $C_{11}H_{23}NO_2S \cdot HCl$: C, 48.97; H, 8.96; N, 5.19; Cl, 13.14; S, 11.89. Found: C, 49.29; H, 8.89; N, 5.39; Cl, 13.51; S, 12.15.

L-1-Tosyl-5-*N'*-*tert*-butyl carboxamido-2-pyrrolidone (XX).—Tosyl-L-glutamyl dichloride[85,86] (5.00 g., 0.015 mole) was dropped into a stirred solution of 3.00 g. (0.045 mole) of *tert*-butylamine[87] in 50 ml. of chloroform. Heat evolution was noted. The resulting mixture was taken to dryness under vacuum and the residual oil washed with water. The oil was dissolved in ether and the ether evaporated. Crystallization ensued yielding 3.44 g. (69%), m.p. 158–176° with softening at 138°. The product was twice recrystallized from 100 ml. of 50% ethanol giving 3.09 g. (62%) of long prisms, m.p. 184.5–185.5°, $[\alpha]^{25}D$ −26° (*c* 1, methanol).

Anal. Calcd. for $C_{16}H_{22}O_4N_2S$. C, 56.78; H, 6.57; N, 8.28; S, 9.48. Found: C, 56.42; H, 6.55; N, 8.21; S, 9.77.

All attempts to remove the *N*-*tert*-butyl group using a variety of acids failed.

Carbobenzoxy-DL-phenylalanine-*N*-*tert*-butylamide (XXI).—The solution resulting from 8.97 g. (0.030 mole) of carbobenzoxy-DL-phenylalanine,[80,81] 6.18 g. (0.030 mole) of dicyclohexylcarbodiimide, 2.19 g. (0.03 mole) of *tert*-butylamine and 20 ml. of tetrahydrofuran was allowed to stand at room temperature overnight; 3 ml. of acetic acid was then added and 30 min. later 6.43 g. of dicyclohexylurea (theory, 6.73 g.) was filtered off. The residue was taken to half-volume under an air stream. The residue was washed with aqueous bicarbonate solution, *N* sulfuric acid, and water. A gum was obtained that hardened overnight; 5.76 g. (54%), m.p. 104–120°.

The solid was dissolved in 200 ml. of hot isopropyl ether, treated with charcoal and concentrated to 100 ml. A solid (A) appeared and was collected; wt. 1.2 g., m.p. 131–145°. The filtrate was concentrated to 50 ml. giving 2.2 g., m.p. 120.5–123° (B). The (A) material was now insoluble in isopropyl ether and was further purified by boiling with that solvent; m.p. 140–149°. Recrystallization from 20 ml. of ethyl acetate gave 0.44 g. (4%) of *N*-*t*-butyl carbobenzoxy-DL-phenylalaninate, m.p. 151.5–152.5°. The structure was confirmed by synthesis from carbobenzoxy-DL-phenylalanine and *t*-butylamine using N,N'-carbonyldiimidazole.

Anal. Calcd. for $C_{21}H_{26}O_3N_2$: C, 71.16; H, 7.39; N, 7.90. Found: C, 71.26; H, 7.77; N, 8.31.

Only impure materials could be obtained from the mother liquors. The (B) material was recrystallized three times from isopropyl ether giving 1.55 g. (10%) of *N*-(carbobenzoxy-DL-phenylalanyl)-*N*,*N'*-dicyclohexylurea, m.p. 122–123°.

Anal. Calcd. for $C_{30}H_{39}O_4N_3$: C, 71.10; H, 7.83; N, 8.18. Found: C, 70.94; H, 7.59; N, 7.90.

Attempted Preparation of *tert*-Butyloxycarbonyl-DL-alanine *N*-*tert*-Butylamide and Carbobenzoxy-L-leucine *N*-*tert*-Butylamide (XXII).—Attempts to repeat the phenylalanine procedure with *tert*-butyloxycarbonyl-DL-alanine[43] and carbobenzoxy-L-leucine[75] gave only their acylureas. The latter was an oil that defied further purification. The former, a solid, was recrystallized from ethyl acetate; m.p. 163.5–164°, yield 16%. It was *N*-(*tert*-butyloxycarbonyl)-DL-alanyl-*N*,*N'*-dicyclohexylurea.

Anal. Calcd. for $C_{21}H_{37}O_4N_3$: C, 63.77; H, 9.43; N, 10.62. Found: C, 63.94; H, 9.32; N, 10.30.

It was noted that infrared spectra of all the acylureas gave a very pronounced CH_2– peak at 6.85 μ.[88] On going back to earlier work where acylureas had been obtained we observed that their infrared spectra also had strong 6.85 μ bands.

***tert*-Butyl Carbobenzoxy-*N*$^{\beta}$-*tert*-butyl-L-asparaginate and *tert*-Butyl Carbobenzoxy-L-asparaginate (XXIII).**—*N*-Carbobenzoxy-β-cyano-L-alanine[66] (21.5 g., 0.086 mole) was added to 500 ml. of methylene chloride which contained 30 ml. of concentrated sulfuric acid. Gaseous isobutylene was bubbled into the solution until it was separated. The solution was stored at room temperature in a stoppered flask for a period of 20 hours. The material was washed with 500 ml. of a 5% sodium bicarbonate solution and the organic layer separated and dried. Evaporation of the methylene chloride solution yielded 19.5 g. of crystals. These were recrystallized from diisopropyl ether to yield 10 g. of crystals. These were recrystallized from diisopropyl ether to yield 10 g. (32%) of *tert*-butyl carbobenzoxy-*N*$^{\beta}$-*tert*-butyl-L-asparaginate, m.p. 139–140°, $[\alpha]^{25}D$ −8° (*c* 1.00, dimethylformamide).

Anal. Calcd. for $C_{20}H_{30}O_5N_2$: C, 63.47; H, 7.99; N, 7.40. Found: C, 63.44; H, 8.26; N, 7.39.

This compound on reduction yielded the free base (see Table II).

The diisopropyl ether filtrate was evaporated to dryness leaving a gummy residue. This was treated with hydrogen peroxide (30%) according to the procedure of Liberek.[89] An additional 1.3 g. of impure *tert*-butyl carbobenzoxy-*N*$^{\beta}$-*tert*-butyl-L-asparaginate was isolated together with 3.6 g. of crystalline *tert*-butyl carbobenzoxy-L-asparaginate. The latter compound was recrystallized from methylcyclohexane with a 50% recovery (net yield 6.5%). The compound melted at 107–108.5°, lit.[66] m.p. 70°; $[\alpha]^{26}D$ −12° (*c* 1.42, in ethanol).

Anal. Calcd. for $C_{16}H_{22}N_2O_5$: C, 59.61; H, 6.88; N, 8.69. Found: C, 59.94; H, 7.01; N, 8.82.

By converting carbobenzoxy-L-asparagine[90] to its silver salt and subsequently treating[47] the silver salt with *tert*-butyl iodide,[87] a 12% yield of *tert*-butyl carbobenzoxy-L-asparaginate was obtained, m.p. 105–106°. The compound gave a similar analysis.

Two attempts to prepare *tert*-butyl L-asparaginate using the general method of Roeske[45] failed.

(85) J. Swan and V. du Vigneaud, *J. Am. Chem. Soc.*, **76**, 3110 (1954).
(86) R. J. Stedman, *ibid.*, **79**, 4691 (1957).
(87) Purchased from Eastman Distillation Products, Rochester, N. Y.
(88) L. J. Bellamy, "The Infra-red Spectra of Complex Molecules," John Wiley and Sons, Inc., New York, N. Y., 1960, p. 20.
(89) B. Liberek, *Chemistry & Industry*, 987 (1961).
(90) M. Bergmann and L. Zervas, *Ber.*, **65**, 1192 (1932).

Sec. 2. Removal of Protecting Groups.—The conditions for removal of the *tert*-butyl ether group were studied using the *tert*-butyl esters of the *tert*-butoxyamino acids. Two drops of sample was treated with 3 drops of reagent. After an allotted time the reaction was stopped by the addition of an excess of pyridine. The results determined by chromatography of the reaction mixture either on Whatman No. 1 paper using the solvent system 1-butanol–acetic acid–water, 4:1:5, (BAW), or on silica gel plates (thin layer) using the solvent system *sec*-butyl alcohol–3% ammonia, 3:1 (BAM).

Ex. A. Removal of *tert*-Butyl Groups from H·ser(*O*-*t*-Bu)·-*O*-*t*-Bu(DL) (III).—Using ninhydrin in butanol as the detecting reagent, the R_f values in the BAW system were: H·ser·OH-(DL), 0.17; H·ser·O-*t*-Bu(DL), 0.70; H·ser(O-*t*-Bu·OH(DL) 0.72; H·ser(O-*t*-Bu)·O-*t*-Bu(DL) 0.89. Since the second and third compounds could not be satisfactorily separated, the BAM system was used. The results are shown in Table III.

TABLE III

PRODUCTS FROM H·ser·(O-*t*-Bu)·O-*t*-Bu(DL), R_f VALUES (BAM)

Treatment at 25°	H·ser·OH	H·ser(O-*t*-Bu)·OH	H·ser·O-*t*-Bu	H·ser(O-*t*-Bu)·O-*t*-Bu
Standards	0.04	0.17	0.48	0.60
HBr/HOAc (5 min.)	.02	.16	.48	.60
HI (57%) (5 min.)	.05	.19	.46	.62
HCl/$CHCl_3$ (5 min.)	.04	.16	.44	.59
HBr/HOAc (30 min.)	.04			
HCl/$CHCl_3$ (30 min.)	.03	.17	.53	.68

Thus, the best method of removing both protecting groups appears to be hydrogen bromide in acetic acid for a 30-minute period.

Ex. B. Removal of *tert*-Butyl Groups from H·cy(S-*t*-Bu)·O-*t*-Bu(L) (XX).—The R_f values in the BAW system for the ester-thioether and related compounds were: H·cySH·OH(L), 0.06; H·cy(S-*t*-Bu)·OH(L), 0.78; H·cy(S-*t*-Bu)·O-*t*-Bu(L), 0.89. The ester-thioether was treated, as described in ex. A, with perchloric acid (70%), hydriodic acid (57%), hydriodic acid in acetic acid, and trifluoroacetic acid (TFA). The chromatograms were treated with ninhydrin, then Feigl reagent,[91] the latter reagent showing the presence of a free SH- group. Although all of the reagents except TFA yielded free cysteine, none of the reactions went to completion. The latter reagent gave a product with an R_f value of 0.70 which was positive to Feigl reagent. This has been tentatively identified as H·cySH·O-*t*-Bu(L), since a spot which was ninhydrin positive with the same R_f value was found in the starting base (ex. XX).

The R_f values of the cysteine derivatives in BAM were determined as: H·cySH·OH(L), 0.05 H·cy(S-*t*-Bu)·OH(L), 0.19; H·cy(S-*t*-Bu)·O-*t*-Bu(L), 0.72; H·cySH·O-*t*-Bu, 0.62. Pure compounds were used, except the last value was tentatively determined from a very weak ninhydrin-positive spot which appeared in the distillate of the original preparation. This was eliminated if the base was initially purified as the hydrochloride salt. In addition to the previous reagents, three additional ones were tested: hydrogen chloride in chloroform, hydrogen bromide in nitromethane and perchloric acid (70%) dissolved in glacial acetic acid. A second perchloric acid treatment was continued for an hour.

Development of color on the chromatograms by ninhydrin as well as subsequent treatment with chlorine and potassium iodide–tolidine reagent[92] indicated that no reaction had gone to completion. However, perchloric acid treatment for an hour gave the best results with H·cySH·OH(L) forming, and only one ninhydrin-positive spot remaining. This corresponded to H·cy-(S-*t*-Bu)·OH(L). An unidentified spot (tolidine positive) remained with an R_f of 0.39.

Ex. C. Removal of *tert*-Butyl Groups from H·tyr(O-*t*-Bu)-·O-*t*-Bu(L) (II).—The ester-ether was treated as previously described with hydrogen bromide in acetic acid, hydrobromic acid (40%), hydrogen chloride in chloroform, and *p*-toluenesulfonic acid. All reagents removed both *tert*-butyl groups. The BAW system was used, the spots being detected with ninhydrin; R_f 0.59 for tyrosine and 0.94 for H·tyr(O-*t*-Bu)·O-*t*-Bu(L).

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(91) Fritz Feigl, "Qualitative Analysis of Spot Tests," 2nd English Ed., Nordeman Pub. Co., Inc., New York, N. Y., 1939, pp. 195 and 291.

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[CONTRIBUTION FROM THE CHANDLER LABORATORIES OF COLUMBIA UNIVERSITY, NEW YORK 27, N. Y.]

The Enamine Alkylation and Acylation of Carbonyl Compounds

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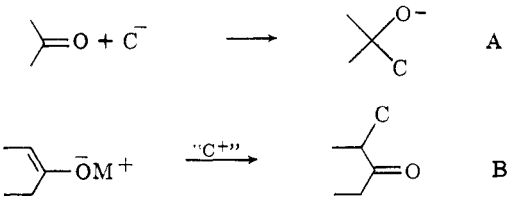
The enamine alkylation and acylation of carbonyl compounds is discussed with regard to the preparation of enamines, their alkylation with electrophilic olefins, their alkylation with alkyl halides and finally their acylation with acid chlorides. This new synthetic method is remarkable by its mildness and by the ease with which monoalkylation or acylation can be achieved.

Introduction

In 1954, we introduced a new and relatively general synthetic method for the acylation and alkylation of carbonyl compounds.[1,2] In the ensuing years the usefulness of the new reaction has been abundantly demonstrated by work in this Laboratory and elsewhere, and well over ninety papers have appeared since our initial publications.[3] A progress report on our own further work in this field has also been given.[4] Our interest in devising new methods for the formation of the carbon–carbon bond stems from the fact that there is a relative scarcity of reactions that will accomplish this fundamental synthetic operation. In fact, a high proportion of the carbon–carbon-forming reactions of interest in complex syntheses belong to two categories: the addition of a carbanion to a carbonyl group (aldol, Grignard, metal acetylide reactions, etc.; *cf.* A) and the reaction of the enolate derived from a carbonyl group with an electrophilic carbon (aldol, Claisen and related reactions, Michael reaction, alkylation of metal enolates, etc.; *cf*, B).



Reactions of type B, although of considerable synthetic importance, suffer from a number of serious limitations which we will illustrate using the alkylation of enolates and the related Michael reaction. Two major difficulties are: (1) the necessity, particularly in the case of alkylation, of using a strong base (*e.g.*, amide ion, triphenylmethide ion, *t*-alkoxides) to transform the carbonyl compound into its anion; (2) the proton transfer reaction between the alkylated ketone formed initially and the unreacted enolate ion. The first problem is illustrated by, *e.g.*, the self-condensation of cyclopentanone by bases under conditions of the

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(3) The literature on enamines is reviewed in a chapter by J. Szmuszkovicz in a forthcoming volume of "Advances in Organic Chemistry," Interscience Publishers, Inc., New York, N. Y.

(4) XVIth National Organic Symposium Abstracts, Seattle, Wash., June, 1959, pp. 44 ff.