Nmr (in $CDCl_3$) showed signals at τ 8.60 (s, 9 H, *tert*-Bu), 7.90 (s, 3 H, C_3 CH_3), 7.0 and 6.58 (2d, 2 H, H_2), 5.14 (d, 1 H, C_6H), 4.67 (d, 1 H, α-CH), 4.34 (q, 1 H, C_7 H), 3.91 (d, 1 H, amide NH), and 2.69 (s, 5 H, aromatic H). Electrometric titration (in 66% aqueous DMF) gave a pK_a of 5.7 and an apparent molecular weight of 500 (calcd 448).

Anal. Calcd for $C_{21}H_{25}N_3O_6S$: C, 56.37; H, 5.63; N, 9.39. Found: C, 56.18; H, 5.80; N, 9.10.

7-(D-Amino-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylic Acid (7). Method A.—6 (3.9 g, 6.0 mmol) was dissolved in 200 ml of 90% aqueous formic acid. The solution was cooled in an ice–H_2O bath. Zinc dust (3.9 g, 60 mg-atoms) was added, and the mixture was stirred for 55 min. The zinc was filtered and washed with 40 ml of aqueous formic acid. The filtrate and wash were combined and evaporated *in vacuo*, azeotroping with C_6H_6 to remove the last traces of formic acid. The residue was taken up in 80 ml of H_2O (pH 3.5) and treated with H_2S for 15 min. The precipitated zinc sulfide was filtered with the aid of Filter-Cel; the filtrate (pH 2) was concentrated to about 20 ml, cooled in ice, and adjusted to pH 7 with 50% NaOH. A slight amount of precipitate was removed by filtration. The solution was reacidified to pH 4.5 (isoelectric point of cephalexin) and diluted with 60 ml of MeCN. The crystallized product was pure cephalexin, 500 mg (24% yield).

Nmr (in D_2O–DCl) showed signals at τ 7.88 (s, 3 H, C_3 CH_3), 6.88 and 6.48 (2d, 2 H, C_2 H_2), 5.0 (d, 1 H, C_6 H), 4.53 (s, 1 H, α-CH), 4.29 (d, 1 H, C_7 H), and 2.32 (s, 5 H, aromatic H) and corresponded exactly with that of an authentic sample of cephalexin prepared according to Ryan, *et al.*[5]

In another run, the work-up was altered: The aqueous filtrate, following the zinc sulfide precipitation, was evaporated to dryness *in vacuo*. The residue was dissolved in 60 ml of MeCN by addition of triethylamine dropwise to pH 9. The mixture was filtered to remove insoluble impurities, and the filtrate was back-titrated to pH 6 with 1 *N* HCl. Cephalexin precipitated in 49% yield.

The bioautograph (*Bacillus subtilis* seeded agar plate of a paper chromatogram, developed in 1-butanol–AcOH–H_2O, 3:1:1) showed a single biologically active spot corresponding exactly in mobility and potency to authentic cephalexin at like concentration.

Method B.—Crude 13 was dissolved in 40 ml of MeCN and 6 ml of H_2O and stirred for 90 min in the cold with zinc dust (1.2 g, 18.4 mg-atoms) and 2 ml of concentrated HCl. The mixture was then filtered, and the filtrate was adjusted to pH 4.5 with NH_4OH. A white, crystalline precipitate developed. This was filtered, washed with MeCN, and vacuum dried, weight 2.5 g. Tlc (using MeCN–H_2O, 4:1 system) and an nmr spectrum of this material showed cephalexin as the major component.

Method C.—Crude 14 (from a 10-mmol run of its preparation) was dissolved in 50 ml of MeCN and treated with *p*-toluenesulfonic acid monohydrate (3.8 g, 20 mmol). The reaction solution was stored at room temperature overnight. The solution was cooled for the addition of 10 ml of H_2O and triethylamine to pH 4.8. After immediate precipitation, the product was filtered, washed with cold MeCN, and dried to constant weight in a vacuum dessicator. The over-all yield of cephalexin from 5d has varied between 69 and 74%.

Anal. Calcd for $C_{16}H_{17}N_3O_4S$: C, 55.33; H, 4.93; N, 12.10. Found: C, 55.19; H, 5.19; N, 11.95.

Nmr, ir, and uv spectra were in agreement with those of authentic cephalexin.

Registry No.—**2a**, 19474-19-2; **2b**, 26774-86-7; **3a**, 19474-21-6; **3b**, 28180-78-1; **3c**, 28180-79-2; **4b**, 28180-80-5; **4c**, 28180-81-6; **5d**, 28180-82-7; **5d** *p*-toluenesulfonate salt, 28180-83-8; **5d** 2-naphthalenesulfonic acid salt, 28180-84-9; **5e** *p*-toluenesulfonate salt, 28180-85-0; **6**, 28292-01-5; **7**, 15686-71-2; **9**, 22252-43-3; **10a**, 10209-11-7; **10a** 2,2,2-trichloroethyl ester, 24647-47-0; **10b**, 27255-72-7; **12**, 28292-02-6; **13**, 28180-91-8; **14**, 28180-92-9; 2,2,2-trichloroethyl chloroformate, 17341-93-4; *N*-(2,2,2-trichloroethyloxycarbonyl)-D-α-phenylglycine, 26553-34-4.

Acknowledgment.—The authors gratefully acknowledge Dr. E. H. Flynn for helpful discussions and the contributions made by the following groups to experimental data: Mr. G. M. Maciak and associates for microanalyses, Mr. D. O. Woolf and associates for spectrometry, Mr. C. T. Pugh for bioautography, and Dr. R. J. Simmons for bioassays.

Specific, Reversible Acylation of Free Peptides Containing Lysine[1]

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Received October 1, 1970

Differences in reactivity between α- and ϵ-amino groups makes possible specific N^ϵ-acylation of free peptides containing lysine, in good yield and under simple experimental conditions. Alanyllysylalanine and N^α-, N^ϵ-, and N^α,N^ϵ-diacyl derivatives thereof were synthesized and used as standards. Reaction of the free tripeptide with *tert*-butylazidoformate at pH 7 was primarily at the N^α position. Reaction in pyridine–water–triethylamine was at the N^ϵ position. Reaction with trifluoroacetic anhydride in trifluoroacetic acid yielded only the N^α-acyl product. The two ϵ-amino groups of porcine β-melanotropin can be specifically acylated with *tert*-butylazidoformate in good yield either in water at pH 10.5 or in pyridine–water–triethylamine. Formation of triacyl-β-melanotropin, in which the terminal amino group is also acylated, required extended reaction times and larger excesses of reagent.

In a semisynthetic preparation of the lysine-10 analog of human β-melanotropin (β-MSH), a suitably blocked tetrapeptide azide was reacted with naturally occurring porcine β-MSH.[2] The latter compound contains two ϵ-amino as well as a terminal α-amino group. Although a solution pH of 6.5 was employed to maintain ϵ-amino sites in a protonated, unreactive form, considerable coupling at N^ϵ positions did occur. The present report describes methods to utilize this apparently very high N^ϵ-amino reactivity to effect specific N^ϵ-acylation of free peptides containing lysine.

Free lysine has been the subject of a number of specific derivatization studies. Bezas and Zervas prepared N^ϵ-benzylidine lysine by virtue of product insolubility and rapid precipitation from solution.[3] Weygand and Geiger synthesized N^α-trifluoroacetyllysine with trifluoroacetic anhydride in trifluoroacetic acid;[4] in this case, strong acid so repressed N^ϵ-ammonium–amino

(1) (a) Presented in part at the 2nd American Peptide Symposium, Cleveland, Ohio, Aug 16–19, 1970. (b) This work was supported by the U. S. Public Health Service, Grant No. CA-04679, and the American Cancer Society, Grant P-168.

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Table I
Identification of Tripeptide Derivatives

Amino acid	Ala-Lys-Ala	Dnp Dnp-Ala-Lys-Ala	Dnp Boc-Ala-Lys-Ala	Dnp Tfa-Ala-Lys-Ala	Boc Dnp-Ala-Lys-Ala
Lysine	1.03 (1)[a]	0.08 (0)	0.02 (0)	0.02 (0)	1.04 (1)
Alanine	1.96 (2)	1.00 (1)	2.00 (2)	2.00 (2)	0.97 (1)

[a] Ratios found (theoretical).

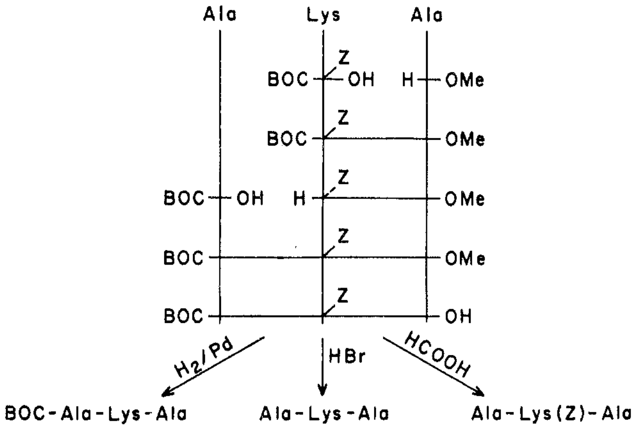

Figure 1.—Synthesis of derivatives of alanyllysylalanine.

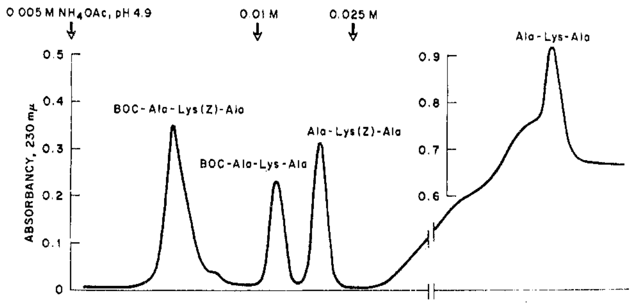

Figure 2.—Resolution of derivatives of alanyllysylalanine on carboxymethyl cellulose.

equilibria that no N[ε]-acylation occurred. LeClerq and Benoiton[5] in a systematic study of conditions for specific acylation of lysine found that nitrophenyl acetate effects N[ε]-acetylation at pH 11. No α-acetylation was observed even with excess reagent and prolonged reaction times. Since both α- and ε-acetylation occur at lower pH, it appears that at pH 11 there is very rapid N[ε]-aminolysis, while hydrolysis is so much faster than α-aminolysis that none of the latter takes place. None of the abovementioned studies was extended to peptides.

Ala-Lys-Ala,[6] used as a model peptide for initial acylation studies, was prepared as shown in Figure 1. This route yielded authentic N^{α}-, N^{ϵ}-, and $N^{\alpha,\epsilon}$-diacyl products as well as free tripeptide. Blocked and partially deblocked dipeptide intermediates were obtained in oily form. Blocked tripeptide ester and acid were obtained in solid, chromatographically and analytically pure form. Partially and fully deblocked tripeptide acids were characterized by electrophoresis, thin layer chromatography, amino acid analysis,[7] and dinitrophenylation.[8] A mixture of all four tripeptide derivatives was resolvable on carboxymethyl cellulose (Figure 2). The order of elution of products indicated that adsorption as well as ion exchange chromatography was occurring, since the α-free compound was retained more strongly than the ε-free product. Electrophoresis in a borate buffer, pH 8.9 (Figure 3), afforded rapid and complete resolution of all four derivatives. In experiments to determine ideal conditions for specific acylation, products were detected by ninhydrin after acid spray to deblock any diacyl derivative formed during the acylation step.

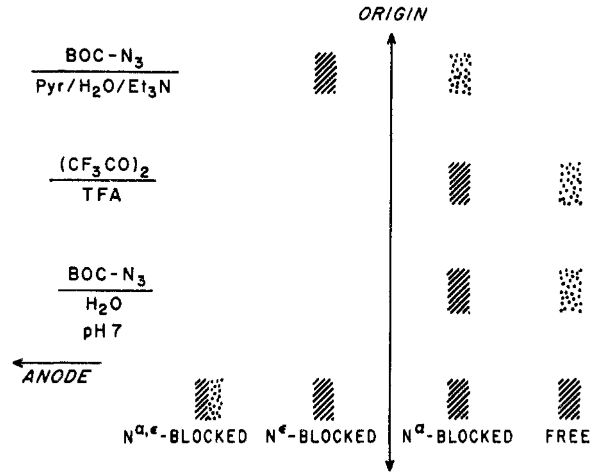

Figure 3.—Electropherogram of derivatives of alanyllysylalanine in borate buffer, pH 8.9.

At pH 7 in water, *tert*-butylazidoformate[9] (2 μl/mg peptide) reacted with Ala-Lys-Ala to give N^{α}-Boc-tripeptide (Figure 3) with no observable N^{ϵ}-acyl product. These results were in contrast to those previously reported[2] in which a blocked tetrapeptide azide coupled to β-MSH both at α- and ε-amino sites, even at lower pH. Exposure of Ala-Lys-Ala in trifluoroacetic acid to trifluoroacetic anhydride also afforded N[α]-blocked material (Figure 3) as described with free lysine.[4] Boc- and Tfa-tripeptides were characterized further by dinitrophenylation and amino acid analysis, which confirmed that α-acylation occurred (Table I). These analyses were performed on crude reaction products, indicating the high yields and degree of specificity of the acylation reactions described.

Similar acylation experiments with naturally occurring porcine β-MSH[10] did not produce similar results. At pH 7 even after 1.5 hr, very little acylation occurred (Figure 4). After 1 hr at pH 10.5 in water, *tert*-butylazidoformate (2 μl/mg peptide) and β-MSH react to form a new ninhydrin positive product in high yield

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TABLE II
IDENTIFICATION OF β-MSH DERIVATIVES

Amino acid	β-MSH[c]	β-MSH, dinitrophenylated[b]	$N^{\alpha},N^{\epsilon},N'^{\epsilon}$-Tri-Boc-β-MSH, dinitrophenylated[b]	$N^{\epsilon},N'^{\epsilon}$-Di-Boc-β-MSH, dinitrophenylated[b]
Lysine	2.09 (2)[a]	0.01 (0)	1.95 (2)	1.98 (2)
Aspartic acid	1.95 (2)	1.05 (1)	1.90 (2)	1.15 (1)
Glutamic acid	2.05 (2)	1.80 (2)	2.03 (2)	1.85 (2)
Glycine	2.00 (2)	2.12 (2)	1.95 (2)	1.95 (2)

[a] Ratios found (theoretical). [b] Products were exposed to fluorodinitrobenzene and then acid hydrolyzed and analyzed quantitatively. [c] The sequence of porcine β-MSH is Asp-Glu-Gly-Pro-Tyr-Lys-Met-Glu-His-Phe-Arg-Trp-Gly-Ser-Pro-Pro-Lys-Asp: J. I. Harris and P. Roos, *Nature*, **178**, 90 (1956).

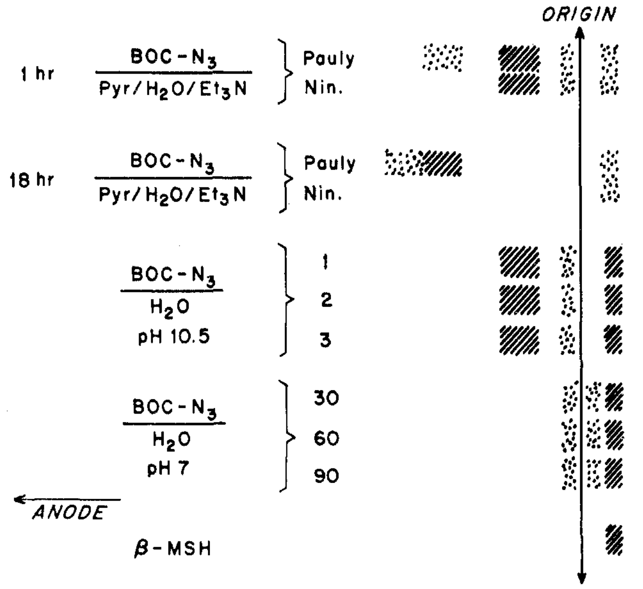

Figure 4.—Electropherogram of derivatives of β-melanotropin in pyridine acetate buffer, pH 6.5.

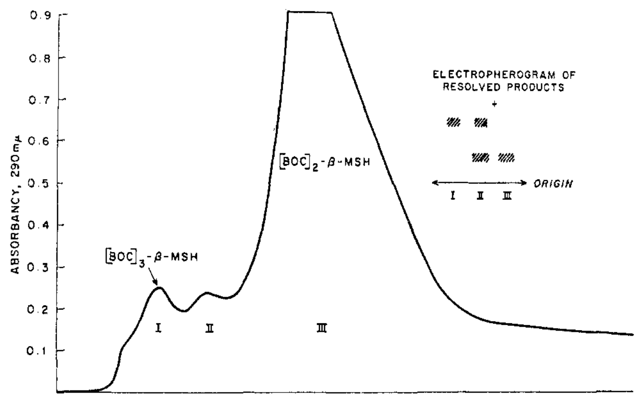

Figure 5.—Resolution of derivatives of β-melanotropin on carboxymethyl cellulose.

(Figure 4). Characterization by dinitrophenylation and amino acid analysis (Table II) following purification by chromatography on carboxymethyl cellulose (Figure 5) showed the product to be $N^{\epsilon},N'^{\epsilon}$-di-Boc-β-MSH; both lysines but only one aspartyl residue were recovered. The same product was produced after 1 hr in pyridine–water–triethylamine 10:10:1. On standing overnight with double amounts of *tert*-butylazidoformate, 4 μl/mg peptide, in pyridine–water–triethylamine, β-MSH is transformed into ninhydrin negative $N^{\alpha},N^{\epsilon},N'^{\epsilon}$-tri-Boc-β-MSH (Figure 4). This product was also characterized by dinitrophenylation and amino acid analysis (Table II). Both lysyl residues and the amino-terminal aspartyl residue are recovered in this case, indicating that all amino moieties are blocked. Exposure of Ala-Lys-Ala to *tert*-butylazidoformate, 2 μl/mg peptide, for 1 hr in the same pyridine buffer also afforded specific N^{ϵ}-acylation in good yield (Figure 3, Table I).

Thus the relatively low reactivity of *tert*-butylazidoformate, coupled with high nucleophilicity of N^{ϵ}-amino groups, appears to afford direct preparation of N^{ϵ}-acyl derivatives of lysine-containing peptides in reasonable yield and under simple reaction conditions. This technique, designed to allow semisynthetic studies with naturally occurring peptides obtained from tryptic hydrolysates, may also find some utility in totally synthetic methodology as well. The procedure makes possible stepwise Edman degradation of naturally occurring lysine-containing peptides for purposes of structure–activity studies, since formation of the stable N^{ϵ}-phenylthiocarbamyl derivatives of lysine can be blocked reversibly. Finally, specific N^{ϵ}-acylation with stable blocking groups may be useful for sequence determination by subtractive Edman degradation techniques.[11] In the usual procedure, recoveries of lysine are low, often making difficult an unequivocal determination of sequence of lysine-containing peptides. Stable N^{ϵ}-acyl derivatives may also be ideal substrates in solid phase Edman degradation techniques.[12]

Experimental Section

Materials and Methods.—All solvents were reagent grade and redistilled; triethylamine (Eastman) was distilled over potassium hydroxide pellets; *tert*-butyleazidoformate (Pierce) was shaken with powdered calcium carbonate prior to use; dicyclohexylcarbodiimide (Eastman), trifluoroactic acid and anhydride (Eastman), 97% formic acid (Aldrich), and fluorodinitrobenzene (Eastman) were all used directly.

Amino acids were purchased from Mann Laboratories; Ala-OMe·HCl was prepared by the method of Brenner and Huber,[13] Boc-Lys(Z) by that of Anderson and McGregor,[14] and Boc-Ala by that of Schwyzer, *et al.*[15]

Thin layer chromatography (tlc) was performed in a sandwich-type apparatus in two systems: system 1, chromar 500 (Mallinckrodt) with chloroform–methanol 95:5; system 2, chromogram, cellulose (Eastman) with butanol–acetic acid–water 4:1:5. Electrophoresis on Whatman 3-mm paper was performed in two buffers: buffer 1, 0.02 *M* sodium borate, pH 8.9; buffer 2, pyridine–acetic acid–water 100:900:4, pH 6.5, in a Savant LT-2A tank. Melting points, uncorrected, were measured on a Thomas-Hoover apparatus. Optical rotations were taken with an O. C. Rudolph and Sons polarimeter, Model 70. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory. Amino acid analyses of peptide hydrolysates prepared as described[7] were made with a Beckman Analyzer, Model 120B.

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For dinitrophenylation,[8] 3 mg of peptide and 10 μl of fluorodinitrobenzene were dissolved in 1 ml 5% aqueous triethylamine, stirred in the dark for 4 hr, and evaporated to dryness; aliquots of product were then hydrolyzed for analysis.

N^{α}-*tert*-Butyloxycarbonyl-N^{ϵ}-benzyloxycarbonyllysylalanine Methyl Ester (a).—To an ice-cold solution of 1.6 g of Ala-OMe·HCl (11.3 mmol) and 1.5 ml of triethylamine in chloroform (10 ml) was added 4.0 g of Boc-Lys(Z)[14] (10.3 mmol) in chloroform (10 ml), followed by a cold solution of 2.12 g of DCC[16] (10.3 mmol) in chloroform (10 ml). After 0.5 hr in the cold and 3 hr at room temperature, the mixture was filtered and the filtrate chilled and extracted three times each with cold 0.05 *N* HCl, water, saturated sodium bicarbonate, and water, dried over magnesium sulfate, and evaporated *in vacuo:* yield 4.4 g (91%); homogeneous on tlc, R_f 0.94, system 1; ultraviolet (uv) positive and ninhydrin negative. Amino acid analysis: Ala, 0.93; Lys, 1.07.

N^{ϵ}-Benzyloxycarbonyllysylalanine Methyl Ester Hydrochloride (b).—Dipeptide a, 1.5 g (3.2 mmol), was dissolved in 4 *N* methanolic HCl (45 ml), left at room temperature for 1 hr, and evaporated four times *in vacuo* with methanol: yield 1.24 g of oil (97%); homogeneous on tlc, R_f 0.0, system 1; uv and ninhydrin positive.

N^{α}-*tert*-Butyloxycarbonylalanyl-N^{ϵ}-benzyloxycarbonyllysylalanine Methyl Ester (c).—To an ice-cold solution of 1.2 g of dipeptide ester b (3 mmol) and triethylamine (0.5 ml) was added 0.57 g of Boc-Ala[15] (3 mmol), followed by 0.62 g of DCC (3 mmol). After standing 0.5 hr in the cold and overnight at room temperature, the mixture was filtered and the filtrate treated as described for peptide a, yield 1.08 g. Traces of side product were removed by extraction with ether: yield 0.9 g (56%); mp 132–136°; $[\alpha]^{19}D$ −38.5° (*c* 1, ethanol); homogeneous on tlc, R_f 0.42, system 1; uv positive and ninhydrin negative. Amino acid analysis: Ala, 1.96; Lys, 1.03.

Anal. Calcd for $C_{26}H_{40}N_4O_8$: C, 58.2; H, 7.51; N, 10.4. Found: C, 58.6; H, 7.50; N, 10.4.

N^{α}-*tert*-Butyloxycarbonylalanyl-N^{ϵ}-benzyloxycarbonyllysylalanine (d).—To a solution of 1 g of tripeptide ester c (1.8 mmol) in 10 ml of methanol, 2.8 ml of 1 *N* sodium hydroxide (aqueous) was added in a dropwise manner. The resultant oily suspension was stirred at room temperature for 1.5 hr, diluted with 50 ml of water, and extracted with ethyl acetate. The aqueous phase was cooled to 0°, acidified to pH 3 with cold 1 *N* hydrochloric acid, extracted into fresh ethyl acetate, and washed with cold water until washings were neutral. The organic phase was then dried over magnesium sulfate and evaporated *in vacuo*. The residue was extracted with ether, yield (insoluble residue) 0.76 g. On standing an additional 85 mg precipitated from the ethereal mother liquor: total yield 0.84 g (89%); mp 94–97°; $[\alpha]^{19}D$ −29.6° (*c* 1, ethanol); homogeneous on tlc, R_f 0.28, system 1; uv positive and ninhydrin negative. Amino acid analysis: Ala, 2.03; Lys, 0.97.

Anal. Calcd for $C_{25}H_{38}N_4O_8$: C, 57.5; H, 7.32; N, 10.7. Found: C, 57.3; H, 7.60; N, 10.5.

Alanyllysylalanine Dihydrobromide (e).—Blocked tripeptide d (0.52 g, 1 mmol) was dissolved in acetic acid saturated with hydrobromic acid (4 ml), left at room temperature for 1 hr, and precipitated with ether, and the precipitate was washed exhaustively with ether. The product was stored *in vacuo* over sodium hydroxide pellets: yield 0.41 g (91%); mp 162–165°; $[\alpha]^{19}D$ −26.2° (*c* 2, 0.5 *N* hydrochloric acid) (as *crystalline* monohydrochloride,[6] $[\alpha]^{23}D$ −42.5°); trace of second component on tlc, major component R_f 0.30, system 2; uv negative and ninhydrin positive; homogeneous on electrophoresis, buffer 1, ninhydrin positive. Amino acid analysis: Ala, 1.90; Lys, 1.07. Amino acid analysis after dinitrophenylation showed only alanine.

Alanyl-N^{ϵ}-benzyloxycarbonyllysylalanine Monoformate.—Blocked tripeptide d, 25 mg (0.51 mmol), was dissolved in 1 ml of 97% formic acid and left for 1 hr at room temperature.[17] The mixture was evaporated *in vacuo*, taken up in water, and lyophilized: yield 21 mg (91%); mp 209–213°; $[\alpha]^{19}D$ −23.5° (*c* 0.85, 0.005 *M* ammonium acetate, pH 4.9); traces of starting material and free tripeptide on tlc, major component R_f 0.84, system 2; uv and ninhydrin positive; single component on electrophoresis, buffer 1, ninhydrin positive. Amino acid analysis: Ala, 2.00; Lys 1.00. Amino acid analysis after dinitrophenylation: Ala, 0.98; Lys, 1.02.

N^{α}-*tert*-Butyloxycarbonylalanyllysylalanine.—Blocked tripeptide d, 26 mg (0.5 mmol), was dissolved in methanol (10 ml) neutralized with triethylamine to an apparent pH of 7 using pH indicator paper, hydrogenated over a palladium catalyst in a stream of hydrogen for 2.5 hr, and filtered, and the filtrate was evaporated *in vacuo* and the residue taken up in water and lyophilized: yield 16 mg (84%); mp 110–114°; $[\alpha]^{19}D$ −34.9° (*c* 0.3, 0.005 *M* ammonium acetate, pH 4.9); homogeneous on tlc, R_f 0.75, system 2; ninhydrin positive; homogeneous on electrophoresis, buffer 1, ninhydrin positive. Amino acid analysis: Ala, 1.93; Lys, 1.20. Amino acid analysis after dinitrophenylation showed less than 1% lysine relative to alanine.

Reaction of Alanyllysylalanine with *tert*-Butylazidoformate. 1. In Water, pH 7.—Peptide e, 2 mg, in 2 ml of water was titrated to pH 7 with a Radiometer pH-Stat and reacted with *tert*-butylazidoformate (4 μl) under nitrogen at constant pH and with vigorous stirring. After 0.5 hr the mixture was extracted with ether and the aqueous phase lyophilized. On electrophoresis in buffer 1, the major product exhibited the same mobility as authentic Boc-Ala-Lys-Ala (Figure 3). Results of amino acid analysis after dinitrophenylation confirm that N^{α}-acylation occurred (Table I).

2. In Pyridine–Water–Triethylamine.—A solution of peptide e (2 mg) and *tert*-butylazidoformate (4 μl) in pyridine–water–triethylamine 10:10:1 was kept at room temperature 1 hr and extracted with 1 ml of ether and the aqueous phase was evaporated *in vacuo* and lyophilized. On electrophoresis in buffer 1, the major component exhibited the same mobility as authentic Ala-Lys(Z)-Ala (Figure 3). Results of amino acid analysis after dinitrophenylation confirm that N^{ϵ}-acylation occurred (Table I).

Reaction of Porcine β-Melanotropin with *tert*-Butylazidoformate. 1. In Water, pH 10.5.—β-Melanotropin (2 mg) in 2 ml of water was titrated to pH 10.5 with a Radiometer pH-Stat and reacted with 4 μl of *tert*-butylazidoformate at constant pH, with vigorous stirring and under nitrogen for 0.5, 1, or 1.5 hr with similar results. The mixture was extracted with 2 ml of ether and the aqueous phase lyophilized. Results of electrophoresis in buffer 2 are shown in Figure 4. The product was ninhydrin positive.

2. In Pyridine–Water–Triethylamine.—A solution of β-MSH (40 mg) and *tert*-butylazidoformate (80 μl) in pyridine–water–triethylamine 10:10:1 (1 ml) was stored at room temperature for 1 hr and extracted with 1 ml of ether and the aqueous phase evaporated *in vacuo*. The residue was chromatographed on a 1.2 × 90 cm column of carboxymethyl cellulose, Whatman CM-52 with a 0.005 *M* ammonium acetate buffer, pH 4.9, using a flow rate of 0.1 ml/min (Figure 5). The major component after lyophilization, 24 mg (60%), was electrophoretically homogeneous in buffer 2 (Figure 5, insert). Amino acid analysis after dinitrophenylation (Table II) identified the product to be $N^{\epsilon},N'^{\epsilon}$-di-Boc-β-MSH.

Preparation of Fully Acylated β-Melanotropin.—A solution of 5 mg of β-MSH and 25 μl of *tert*-butylazidoformate in 1 ml of pyridine–water–triethylamine 10:10:1 was left at room temperature overnight. The mixture was evaporated *in vacuo*, taken up in water, and lyophilized. Results of electrophoresis in buffer 2 are shown in Figure 4; the product was ninhydrin negative. Amino acid analysis after dinitrophenylation identified the product to be $N^{\alpha},N^{\epsilon},N'^{\epsilon}$-tri-Boc-β-MSH.

Registry No.—a, 22839-06-1; b, 27909-28-0; c, 27909-29-1; d, 27909-30-4; e, 27909-31-5; alanyl-N^{ϵ}-benzyloxycarbonyllysylalanine monoformate, 27909-32-6; N^{α}-*tert*-butyloxycarbonylalanyllysylalanine, 27909-33-7.

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