tributable to the $-CH_2N{=}CH-$ system, an aromatic region differentiated into 4 H and 5 H parts, and singlet signals appropriate to the substituents. These data are collected in Table II.

Isomerization. Solutions of 0.3 g of imine in 20 ml of 1 N ethanolic sodium ethoxide were heated under reflux (solution temperature 82 °C). Reaction was quenched at a determined time by rapid cooling with cold water and dilution with 20 ml of distilled water. The resulting mixtures were extracted twice with chloroform, and the extracts were washed twice with water and then dried over sodium sulfate.

Analysis. The dried chloroform solutions were evaporated under vacuum and the residue was dissolved in carbon tetrachloride containing 1% Me_4Si. NMR spectra were determined on a Varian T-60 instrument. Addition of a drop of Me_2SO-d_6 or CD_3OD enhanced the resolution of the signals of the pairs of isomers present. The intensities of the methyl signals (where present; see Table II) were compared to obtain the ratios reported in Table III. In general, three to five samples of each imine were used; except for the *p*-dimethylamino pair, for which all samples are reported, only the mean values are shown.

The mixtures with a *p*-chloro substituent were first hydrolyzed by emulsifying with a small amount of methanol–water mixture and heating with 30 ml of 2 N sulfuric acid at 100 °C for 30 min. The cooled mixture was then extracted with ether, the dried extracts were evaporated to dryness, and the residue was taken up in carbon tetrachloride for NMR analysis by comparison of the aldehydic CH signals.

The reliability of the methods was examined by using mixtures of known compositions. For the pair *N*-benzylidene-*p*-methylbenzylamine/*p*-methylbenzylidenebenzylamine, the results follow: known, 59.5/40.5 (found, 59.8/40.2); known, 58.2/41.8 (found, 59.5/40.5); known, 55.9/44.1 (found, 56.1/43.9). For benzaldehyde/*p*-chlorobenzaldehyde mixtures derived from the *p*-chloro tautomeric imines, the results follow: known, 61.1/38.9 (found, 62.0/38.0); known, 62.4/37.6 (found, 63.0/37.0).

References and Notes

(1) P. A. S. Smith and S. E. Gloyer, *J. Org. Chem.*, **40**, 2504 (1975).
(2) C. K. Ingold and C. W. Shoppee, *J. Chem. Soc.*, 1199 (1929).
(3) C. W. Shoppee, *J. Chem. Soc.*, 1225 (1931).
(4) J. W. Baker, W. S. Nathan, and C. W. Shoppee, *J. Chem. Soc.*, 1847 (1935).
(5) D. J. Cram and R. D. Guthrie, *J. Am. Chem. Soc.*, **87**, 397 (1965).
(6) J. W. Baker, "Hyperconjugation", Oxford University Press, London, 1952, p 66.
(7) R. P. Ossorio, *An. Quim.*, **66**, 87 (1970).
(8) F. Feigl and V. Anger, *Anal. Chim. Acta*, **24**, 494 (1961).
(9) H. H. Jaffe, *Chem. Rev.*, **53**, 191 (1953).
(10) W. E. Dennings, "Statistical Adjustments of Data", Wiley, New York, N.Y., 1943.

Occurrence of N-Alkylation during the Acidolytic Cleavage of Urethane Protecting Groups[1a,b]

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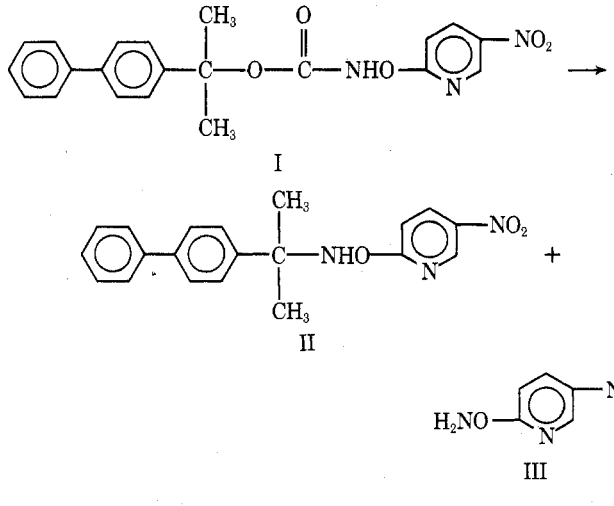
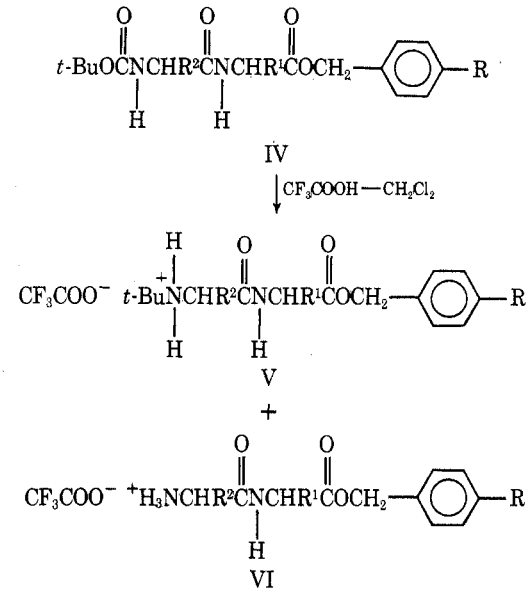
The occurrence of N-alkylation as a side reaction during the acidolytic cleavage of urethane protecting groups by trifluoroacetic acid has been investigated under the conditions of solid phase peptide synthesis. N-Alkylation did not occur when the protecting group was *tert*-butyloxycarbonyl (Boc) as treatment of Boc-Gly-Lys(Z)-resin with 50% CF_3COOH–CH_2Cl_2 did not produce *t*-Bu-Gly-Lys(Z)-resin (<0.05%). This novel side reaction did occur when the protecting group was benzyloxycarbonyl (Z). When Boc-Lys(Z)-resin was treated with 50% CF_3COOH for 14 h (25 °C) the Z group was partially removed and gave rise to 0.6% N^{ϵ}-benzyllysine-resin. The use of a more acid stable N^{ϵ} protecting group (2,4-Cl_2Z) suppressed N-alkylation to less than detectable levels (<0.1%). The acidolytic removal of the benzyloxycarbonyl group from Z derivatives in solution was also studied. Treatment of Z-Gly and Lys(Z) (0.1 M) in refluxing CF_3COOH (30 min) gave 1.1% Bzl-Gly and 3.3% Lys(Bzl), respectively. The addition of 20% anisole gave 0.5% Bzl-Gly and 2.1% Lys(Bzl) from the same Z derivatives. The use of CF_3SO_3H–CF_3COOH–anisole (30 min, 25 °C) allowed the formation of 1.2% Bzl-Gly from Z-Gly and 3.1% Lys(Bzl) from Lys(Z). No N-alkylation could be detected when amino acid resins or free amino acids containing Z protecting groups were cleaved with anhydrous HF.

The most widely used amino protecting groups in peptide synthesis are the benzyloxycarbonyl (Z)[2] and *tert*-butyloxycarbonyl (Boc)[3] groups. A recent addition to this family of urethane-type protecting groups is the relatively acid-labile biphenylisopropyloxycarbonyl (Bpoc)[4,5] group. A report[6] of N-alkylation during the acidolytic cleavage of a Bpoc group from a derivative of hydroxylamine initially prompted the present study as a possible explanation of a rise in background observed with picrate monitoring during solid phase peptide synthesis.[7]

The unexpected formation (20%) of *N*-2-(*p*-biphenylyl)-isopropyl-*O*-(5-nitro-2-pyridyl)hydroxylamine (II) occurred when 2-(*p*-biphenylyl)isopropyl *N*-(5-nitro-2-pyridyloxy)-carbamate (I) was treated with acetic acid in nitromethane.[6] Attack of III by *p*-biphenylyldimethyl carbonium ion could give rise to II. Attack of the carbonium ion on the carbamic acid formed from I, with simultaneous decarboxylation, was also considered a possible route to II.



Our initial interest was focused on the possible occurrence of an analogous reaction with the Boc group under conditions of solid phase peptide synthesis. The formation of a small amount (ca. 0.1–1%) of N^{α}-*tert*-butyl peptide (V) during each

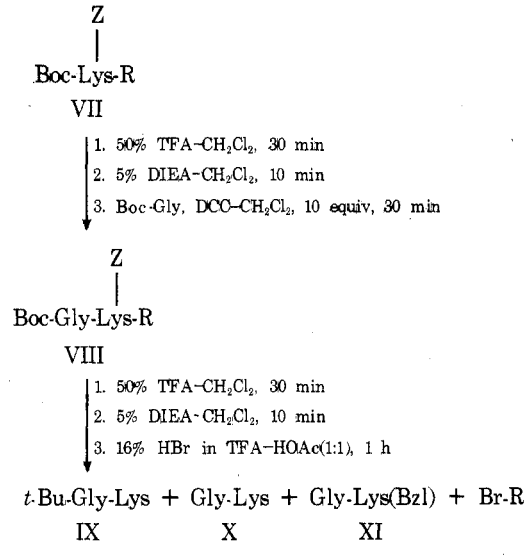


deprotection step is undesirable because it would give rise to terminated chains or N-alkyl peptides. In addition, the production of a variable amount of hindered secondary amines would give corresponding increases in background if a chloride[8] or picrate[9] monitoring method were used to follow the course of a solid phase peptide synthesis.

Results and Discussion

To detect the occurrence of low levels of such side reactions, a sensitive model system employing Boc-Lys(Z)-resin (VII) was devised (see Chart I). The presence of lysine, with α- and

Chart I[a]



[a] Model system for the detection of N$^{\alpha}$-*tert*-butylation and N$^{\epsilon}$-benzylation during solid phase peptide synthesis. TFA, trifluoroacetic acid; DIEA, diisopropylethylamine; HOAc, acetic acid; DCC, *N,N'*-dicyclohexylcarbodiimide; R, ring-brominated oxymethylcopoly(styrene–1% divinylbenzene).

ϵ-amino groups that can react with ninhydrin, allows the detection of reaction products having either the α or ϵ position blocked. For chromatographic reference *t*-Bu-Gly-Lys (IX) was prepared by treating N^{α}-chloroacetyl-N^{ϵ}-Z-L-lysine[10] with *tert*-butylamine followed by decarbobenzoxylation in refluxing TFA.[11] Similarly, Gly-Lys (X) was obtained starting with N^{α}-chloroacetyl-N^{ϵ}-Z-L-lysine and ammonium hydroxide.[10] Compounds IX and X are well resolved on the long column of an amino acid analyzer, thereby allowing the detection of small amounts (≥0.05%) of IX in an overloaded sample containing large amounts of X. The use of an amino acid analyzer for the detection of small quantities of amino acids and peptides in the presence of large amounts of similar compounds has been described elsewhere.[12,13]

Table I. Reaction of Boc-Lys(Z)-R[a] with Cleavage Reagents

Run		Time, h	N^{ϵ}-Bzl-Lys, mol %[b]
1	5% TFA–CH_2Cl_2 (v/v)	14	0
2	50% TFA–CH_2Cl_2 (v/v)	14	0.67
3	TFA	14	2.56
4	16% HBr in TFA–HOAc (1:1)[c]	1	0
5	HF	1	0

[a] R represents ring-brominated oxymethylcopoly(styrene–1% divinylbenzene).[19] Initial substitution of resin was 0.457 mmol Lys/g. The reactions used 0.100–0.200 g of resin in 2–4 ml of cleavage solution and were run at room temperature. [b] The resins obtained from runs 1–3 were washed with CH_2Cl_2, dried, and then hydrolyzed in HCl–C_2H_5COOH. The values obtained for runs 1–3 have been corrected for the production of 0.34 mol % N^{ϵ}-Bzl-Lys which occurred when untreated Boc-Lys(Z)-R was hydrolyzed in HCl–propionic acid. The hydrolysates were analyzed on the short column (0.9 × 7 cm PA-35 sulfonated polystyrene, sodium citrate buffer, pH 7.0, 66 ml/h, 56 °C) of a Beckman 120B amino acid analyzer. The cleavage products from runs 4 and 5 were chromatographed without further treatment. Limit of detection was 0.1 mol %. [c] One volume of 32% HBr–HOAc was added to Boc-Lys(Z)-R that was suspended in one volume of TFA.

The protected dipeptide-resin (VIII) was deprotected, neutralized, and cleaved as indicated in Chart I. Analysis of the cleavage products relative to Gly-Lys (100%) gave Lys (0.16%), and no *t*-Bu-Gly-Lys (<0.05%). The procedure was repeated in the presence of Boc-Gly-OEt (50 equiv), an additional source of *tert*-butyl carbonium ion, during the deprotection step. Again, Lys (0.08%), Gly-Lys (100%), and no *t*-Bu-Gly-Lys (<0.05%) were detected. It was concluded that significant N-alkylation during the acidolytic cleavage of *tert*-butyloxycarbonyl peptides does not occur.

The possible occurrence of N-benzylation during solid phase peptide synthesis was then investigated. A portion of Boc-Gly-resin was treated with 50% TFA–CH_2Cl_2 containing 40 equiv of benzyl carbamate (Z-NH_2) for 1 h. The resin was washed, dried, and hydrolyzed in HCl–propionic acid.[14] No N-Bzl-Gly[15] (<0.1%) was detected. The occurrence of N$^{\epsilon}$-benzylation was indicated, however, when the cleavage mixture obtained from VIII was examined in more detail. Since Gly-Lys(Bzl) was not eluted on the ion-exchange system used to detect IX and X, a portion of the cleavage mixture was hydrolyzed in HCl–propionic acid[14] and then chromatographed on the short column of the amino acid analyzer at pH 7.0. A broad peak that eluted later than the basic amino acids was observed. An authentic sample of N^{ϵ}-Bzl-Lys[16] eluted at the same position, thereby indicating the presence of Gly-Lys(Bzl) (0.17%) in the cleavage mixture.

Portions of Boc-Lys(Z)-resin (VII) were treated with cleavage reagents containing TFA–CH_2Cl_2, HBr–HOAc–TFA,[17] and HF.[18] The results are given in Table I. Increasing concentrations of trifluoroacetic acid promoted increased formation of N^{ϵ}-Bzl-Lys. The addition of various carbonium ion scavengers to cleavage solutions containing trifluoroacetic acid did not significantly alter the formation of N^{ϵ}-Bzl-Lys from VII (Table II). The use of methanesulfonic acid–trifluoroacetic acid solutions[20] lowered the effect somewhat while

Table II. Reaction of Boc-Lys(Z)-R[a] with Cleavage Reagents Containing Carbonium Ion Scavengers

Run	Reagent	N^{ϵ}-Bzl-Lys, mol %
1	TFA–CH_2Cl_2 (1:1)	0.57
2	TFA–CH_2Cl_2 (1:1); 0.08 M DTT	0.88
3	TFA–CH_2Cl_2–anisole (5:4:1)	0.69
4	TFA–anisole (1:1)	0.40
5	TFA–*m*-xylene (1:1)	0.57
6	0.06 M MSA in CH_2Cl_2	0.47
7	0.06 M MSA in CH_2Cl_2–anisole (9:1)	0.24
8	0.01 M MSA and 0.1 M CF_3COOH in *m*-xylene	0.16
9	1 N HCl in glacial CH_3COOH	0.1
10	4 N HCl in dioxane	0

[a] See footnotes *a–c* of Table I for the materials and conditions used. The reactions were run for 14 h at room temperature and the resin products were cleaved with 16% HBr in TFA–HOAc (1:1). MSA, methanesulfonic acid; DTT, dithiothreitol. Limit of detection was 0.1 mol %.

hydrogen chloride in glacial acetic acid or dioxane depressed the formation of N^{ϵ}-Bzl-Lys to about the level of detectability.

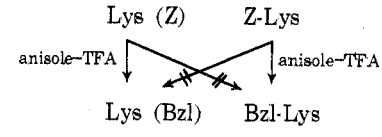
The formation of N^{ϵ}-benzyllysine from N^{ϵ}-benzyloxycarbonyllysine residues during deprotection by acidic reagents is a novel side reaction. It should be noted, however, that it is not the major side reaction that occurs during the deprotection of N^{α}-amino protecting groups in solid phase peptide synthesis. The major side reaction is the N^{ϵ}-decarbobenzoxylation of lysine side chains.[21-23] A calculation using the rate constant (1.9×10^{-6} s^{-1}) obtained for the N^{ϵ}-deprotection of Boc-[Lys(Z)]$_{10}$-Val-resin in 50% TFA–CH_2Cl_2[23] indicates that 9.1% cleavage of N^{ϵ}-benzyloxycarbonyl groups can be expected in 14 h. When VII was treated in 50% TFA–CH_2Cl_2 for 14 h the formation of 0.57% of N^{ϵ}-Bzl-Lys was observed (run 1, Table II). Therefore, about 6.5% of the prematurely N^{ϵ}-decarbobenzoxylated groups gave rise to N^{ϵ}-benzyl side chains. Both of these side reactions are dependent on the concentration of acid present in the deprotection reagent (runs 1–3, Table I) and on the acid stability of the Z group. The use of more acid-stable N^{ϵ}-protecting groups[21-23] has been shown to suppress the undesired deprotection reaction to acceptable levels and was expected to similarly suppress the N-alkylation reaction under the conditions of solid phase peptide synthesis. When Lys(2,4-Cl_2Z)[23] was allowed to stand in 50% TFA–CH_2Cl_2 for 67 h at room temperature only Lys (1.5%) and Lys(2,4-Cl_2Z) (98.5%) were detected. Since very little deprotection occurred, no Lys(2,4-Cl_2Bzl) (<0.1%) could be detected.

The above studies of N-benzylation dealt with the formation of *N*-benzyl amino acids and peptides during conditions which gave only partial deprotection of N^{ϵ}-Z groups. The occurrence of N-benzylation during conditions which allow complete decarbobenzoxylation, namely refluxing in anhydrous trifluoroacetic acid or treatment with CF_3SO_3H–TFA at 25 °C, was next investigated. The use of refluxing TFA for removal of Z groups was originally proposed by Weygand and Steglich[11] and it has found use in peptide synthesis,[24] although cleavage by anhydrous HBr remains by far the most frequently used method for acidolytic decarbobenzoxylations.[25] Samples of N^{α}-Z-Gly and N^{ϵ}-Z-Lys were refluxed in anhydrous TFA (30 min) and analyzed for the presence of free amino acid and *N*-benzyl amino acid. Anisole (20%) was present in a duplicate series of experiments. The results are given in Table III. Lower substrate concentrations reduced but did not entirely suppress N-benzylation in refluxing TFA. The presence of anisole also reduced but did not eliminate N-benzylation in refluxing TFA. When Lys(2,4-Cl_2Z) was refluxed in TFA for 10 h, the presence of Lys (96.2%), Lys(2,4-Cl_2Z) (1.0%), and Lys(2,4-Cl_2Bzl) (2.8%) was observed. In addition, treatment of N^{ϵ}-Z-Lys in refluxing TFA gave Lys and N^{ϵ}-Bzl-Lys; the formation of N^{α}-Bzl-Lys was not observed. Conversely, when N^{α}-Z-Lys was refluxed in TFA no formation of N^{ϵ}-Bzl-Lys was observed. These observations

Table III. N-Alkylation under Conditions of Complete Deprotection of Z-Gly and N^{ϵ}-Z-Lys in Refluxing TFA

Derivative	Concn, M	*N*-Benzyl amino acid,[a] mol %
Z-Gly	1.0	2.10 (0.98)[b]
Z-Gly	0.1	1.12 (0.49)
Z-Gly	0.01	0.96 (0.47)
N^{ϵ}-Z-Lys	0.1	3.26 (2.11)

[a] Z-Gly deprotects to give Gly and N^{α}-Bzl-Gly, while N^{ϵ}-Z-Lys gives Lys and N^{ϵ}-Bzl-Lys. Recoveries of 95–101% were observed. [b] The values in parentheses were obtained from duplicate runs in refluxing TFA containing 20% anisole.



suggest that the formation of *N*-benzyl groups from *N*-benzyloxycarbonyl groups in TFA may proceed via an intramolecular rather than intermolecular pathway.

The test system was also used to evaluate trifluoromethanesulfonic acid, a reagent that was recently proposed for the removal of protecting groups from amino acids and peptides.[26] Trifluoromethanesulfonic acid in methylene chloride or trifluoroacetic acid was reported to resemble HF[18] and boron tris(trifluoroacetate)[27] in its efficiency in cleaving protecting groups from amino acids and peptides. Trifluoromethanesulfonic acid (8.3–9.4 equiv) was added to 0.1 M solutions of Z-Gly and N^{ϵ}-Z-Lys in trifluoroacetic acid containing anisole (2.3–2.6 equiv). Samples were withdrawn at 30 min, quenched with aqueous buffer, and chromatographed on an amino acid analyzer. Z-Gly gave 1.22% of *N*-Bzl-Gly while N^{ϵ}-Z-Lys afforded 3.13% of N^{ϵ}-Bzl-Lys. Therefore, treatment by either trifluoromethanesulfonic–trifluoroacetic acid or refluxing trifluoroacetic acid (Table III) gave rise to N-benzylation during the decarbobenzoxylation of Z-protected amino acids. Pending further investigation, trifluoromethanesulfonic acid cannot be recommended as a general reagent (in lieu of HF, for example) for the removal of Z groups from peptides, although this reagent may prove to be advantageous in selected cases. In contrast, less than 0.1% N-alkylation was observed when Z-Gly, Lys(Z), and Lys(2,4-Cl_2Z) were treated with anhydrous HF (30–60 min, 25 °C). The best way to avoid N-benzylation is to use a substituted Z group which is stable during the multiple deprotections by TFA in a stepwise synthesis but can be removed by HF under conditions which do not cause N-benzylation.

It should be noted that the present investigation dealt with the Boc and Z protecting groups. The possible occurrence of N-alkylation reactions with other urethane-type protecting groups such as *p*-methoxybenzyloxycarbonyl,[28] biphenylisopropyloxycarbonyl,[4,5] and phenylisopropyloxycarbonyl[29] should be of interest to investigators using those protecting groups in peptide synthesis.

In summary, N^{α}-*tert*-butylation was not observed when the Boc protecting group was used in model experiments employing the conditions of solid phase peptide synthesis. A novel side reaction, N-benzylation, was observed when Z groups were removed from Z derivatives of Gly and Lys by trifluoroacetic acid or trifluoromethanesulfonic–trifluoro-

acetic acid. This side reaction was not observed when a more acid-stable Z protecting group was used for lysine.

Experimental Section

Infrared spectra were taken with a Perkin-Elmer Model 237B grating infrared spectrophotometer, using KBr pellets. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Amino acid and peptide analyses were performed on Beckman amino acid analyzers (Models 120B and 121). Elemental analyses were performed by Mr. S. T. Bella of the Microanalytical Laboratory, The Rockefeller University. The solvents used for thin layer chromatography (TLC) (precoated 0.25-mm silica gel G plates, Analtech) were 1-butanol–acetic acid–water (BAW) (4:1:1), 1-butanol–acetic acid–water–pyridine (BAWP) (15:10:3:12), chloroform–methanol–acetic acid (CMA) (17:2:1), and chloroform–methanol–15% aqueous NH_3 (CMN) (4:5:2). Spots were visualized by spraying with 0.2% ninhydrin in 1-butanol and heating. The plates were then exposed to chlorine and sprayed with the *o*-tolidine reagent.

Z-Gly (Eastman) was twice recrystallized from chloroform while ϵ-Z-Lys (Schwarz Bioresearch) was used without further purification. Boc-Lys(Z) was obtained from Fox Chemical Co., and the unsubstituted resin support, a copolymer of styrene–1%-divinylbenzene (200–400 mesh), was purchased from Bio-Rad. The materials and methods for solid phase synthesis were similar to those described elsewhere[13,23,30] but modified as indicated.

N^{α}-Bzl-Gly·HCl. A modification of the method described by Greco et al.[31] for the general preparation of *N*-(substituted benzyl)glycine derivatives was used. Ethyl bromoacetate (11.3 ml, 100 mmol) was cautiously added to a solution of benzylamine (21.8 ml, 200 mmol) in benzene (70 ml). An immediate precipitation of benzylamine hydrobromide occurred. The suspension was refluxed (5 h), cooled, and filtered. The filtrate was evaporated in vacuo yielding a clear, mobile oil (22.1 g) which was refluxed in 6 N HCl (200 ml) for 1.5 h. The resulting solution was evaporated in vacuo until white crystals appeared. The suspension was allowed to stand in the cold for several hours. The crystals were collected, washed with diethyl ether, and dried to give 10.6 g of material, mp 220–225 °C (lit.[32] mp 220 °C). A minor contaminant was detected by TLC (BAWP). A recrystallization was effected from ethanol–diethyl ether, yielding first (5.97 g, mp 223–228 °C) and second (2.58 g, mp 223–226 °C) crops (42% yield) that were homogeneous by TLC, R_f 0.56 (BAWP).

N^{ϵ}-Bzl-Lys·HCl. Catalytic hydrogenation of N^{ϵ}-benzylidene-L-lysine[33] gave rise to the desired product, R_f 0.63 (CMN), in addition to a closely running contaminant, R_f 0.57. Attempts to free the product of the impurity were unsuccessful. The multistep synthesis of N^{ϵ}-benzyl-L-lysine hydrochloride, as described by Benoiton,[16] afforded material that was homogeneous by TLC and ion-exchange chromatography.

N^{α}-Z-N^{ϵ}-(2,4-Cl_2Bzl)-Lys. 2,4-Dichlorobenzaldehyde (1.75 g, 10.0 mmol) and ethanol (5 ml) were added to a solution of N^{α}-carbobenzoxy-L-lysine[33] in 1 N sodium hydroxide (10 ml). The solution was stirred at room temperature for 20 min and then cooled in an ice bath. Sodium borohydride (0.115 g, 3.00 mmol) was added in five portions over a 30-min period. After 15 min, the treatment with 2,4-dichlorobenzaldehyde and sodium borohydride was repeated and the solution was allowed to stir for 4 h. Water (15 ml) was added and the solution was extracted twice with diethyl ether (20 ml). A white solid was obtained when the solution was acidified (HCl) to pH 6.0 and cooled. The solid was collected and recrystallized from methanol–ethyl acetate, yielding 2.24 g (51%) of product: mp 168–170 °C; R_f 0.87 (CMN); $[\alpha]^{24}D$ +1.7° (*c* 2, acetic acid).

Anal. Calcd for $C_{21}H_{24}O_4N_2Cl_2$: C, 57.40; H, 5.50; N, 6.30; Cl, 16.14. Found: C, 57.46; H, 5.49; N, 6.39; Cl, 15.97.

Gly-Lys(Z). This compound was prepared from N^{α}-chloroacetyl-N^{ϵ}-benzyloxycarbonyl-L-lysine and ammonium hydroxide as described by Rao et al.,[10] R_f 0.32 (BAW).

***t*-Bu-Gly-Lyz(Z).** N^{α}-Chloroacetyl-N^{ϵ}-benzyloxycarbonyl-L-lysine[10] (0.933 g, 2.62 mmol) was refluxed in *tert*-butylamine (15 ml, 42 mmol) for 5 h. The *tert*-butylamine was removed in vacuo and the resulting foam was triturated with acetone to give solid material (0.720 g), mp 179–183 °C. Crystallization from methanol–ethyl acetate gave a white solid (0.332 g, 32% yield): mp 196–197 °C; R_f 0.35 (BAW); $[\alpha]^{25}D$ +7.2° (*c* 2, methanol). Carbonyl absorptions were observed in the infrared spectrum at 1686, 1596, and 1530 cm^{-1} and an absorption for *tert*-butyl was observed at 1254 cm^{-1}.

Anal. Calcd for $C_{20}H_{31}N_3O_5$: C, 61.05; H, 7.94; N, 10.68. Found: C, 60.86; H, 7.93; N, 10.68.

A sample of this material was examined by chemical ionization mass spectrometry.[34] The source temperature was 190 °C and the probe temperature approximately 125 °C. Major ions observed were *m/e* 394 (M + 1), 286 ($M^{\cdot+}$ − OCH_2Ph), 147, 91 ($PhCH_2^+$), and 74.

Analyses. Ion-Exchange Chromatography. Ion-exchange chromatography was performed using a Beckman amino acid analyzer (Model 120B or 121) at a flow rate of 66 ml/h and temperature of 56 °C. N^{α}-Bzl-Gly elutes exactly with phenylalanine on the long column (0.9 × 60 cm; AA-15 sulfonated polystyrene) of the analyzer.[15] The ninhydrin color yield of N^{α}-Bzl-Gly is 0.82 relative to Gly. N^{ϵ}-Bzl-Lys emerges from the short column (0.9 × 7 cm; PA-35 sulfonated polystyrene) at 161 min with pH 7.0 sodium citrate buffer. The ninhydrin color yield is 0.70 relative to Lys (28 min).

Samples of Gly-Lys(Z) and *t*-Bu-Gly-Lys(Z) were deprotected in refluxing TFA for 30 min.[11] The TFA was removed in vacuo and the resulting Gly-Lys and *t*-Bu-Gly-Lys trifluoroacetates were used to derive a chromatographic system capable of resolving NH_4Cl, Lys, Gly-Lys, and *t*-Bu-Gly-Lys. These compounds are resolved on the 0.9 × 60 cm column with pH 5.26 buffer.

Compd	Elution time, min	Ninhydrin color yield
Lys	193	1.00
Gly-Lys	235	1.28
NH_4Cl	297	1.29
t-Bu-Gly-Lys	346	0.43

A sample of N^{α}-Z-N^{ϵ}-(2,4-Cl_2Bzl)-L-Lys was decarbobenzoxylated with 32% hydrogen bromide in acetic acid. The HBr and acetic acid were removed in vacuo and the resulting Lys(2,4-Cl_2Bzl)hydrobromide was dissolved in a calibration solution containing Lys and Lys(2,4-Cl_2Z).[23] Lys(2,4-Cl_2Bzl) could not be eluted from the short column (0.9 × 7 cm) of the amino acid analyzer with pH 7 citrate buffer at elevated temperatures (56–90 °C) although Lys and Lys(2,4-Cl_2Z) are readily chromatographed under these conditions.[23] A column of Dowex 50W-X4 sulfonated polystyrene (0.9 × 9 cm), eluted (66 ml/h) with pyridine acetate buffer (0.8 M, pH 5.2) at 25 °C, allowed the resolution of Lys (23 min), Lys(2,4-Cl_2Z) (58 min), and Lys(2,4-Cl_2Bzl) (135 min).

Boc-Lys(Z)-resin.[35] Copoly(styrene–1% divinylbenzene) beads (Bio-Beads SX-1, 200–400 mesh) were washed, chloromethylated (1.0 mmol Cl/g), and brominated as described by Merrifield.[19] The cesium salt of Boc-Lys(Z) was prepared and allowed to react with the ring-brominated chloromethyl resin according to Gisin.[36] A picrate titration[9] after the removal of the Boc group (50% TFA–CH_2Cl_2) indicated a loading of 0.457 mmol Lys/g.

Boc-Gly-Lys(Z)-resin. Formation of *t*-Bu-Gly-Lys. Boc-Lys(Z)-resin (0.200 g, 0.0914 mmol) was placed in a 5-ml reaction vessel[30] and deprotected (30 min) with 50% TFA–$MeCl_2$. The resin was filtered, washed with CH_2Cl_2, and coupled with 10 equiv of Boc-Gly and 10 equiv of DCC in 4 ml of CH_2Cl_2. The coupling mixture was shaken (30 min), filtered, and washed with CH_2Cl_2.

The dipeptide resin was deprotected with 50% TFA–CH_2Cl_2 (30 min), filtered, washed with CH_2Cl_2, neutralized with 5% DIEA in CH_2Cl_2, and washed with CH_2Cl_2. The resin was shaken (30 min) with a cleavage solution containing 2 ml of TFA and 2 ml of 32% HBr in acetic acid.[17] The cleavage solution was filtered and the resin was washed with TFA, TFA–CH_2Cl_2 (1:1), and CH_2Cl_2. The pooled filtrates were evaporated in vacuo. The resulting residue was dissolved in water and subjected to ion-exchange chromatography. Analysis of the cleavage products gave Lys (0.16%), Gly-Lys (99.8%), and no *t*-Bu-Gly-Lys (<0.05%).

The above procedure was repeated with one variation: the Boc-Gly-Lys(Z)-resin was deprotected with 50% TFA–CH_2Cl_2 containing Boc-OEt (50 equiv). Chromatography of the cleavage products gave Lys (0.08%), Gly-Lys (99.9%), and no *t*-Bu-Gly-Lys (<0.05%).

Reaction of Boc-Lys(Z)-resin with Cleavage Reagents. Formation of N^{ϵ}-Bzl-Lys. A. Reaction with TFA–CH_2Cl_2. Solutions (2–4 ml) containing 5% TFA–CH_2Cl_2, 50% TFA–CH_2Cl_2, and 100% TFA were shaken with samples of Boc-Lys(Z)-resin (0.100–0.200 g) for 14 h at room temperature. The resins were filtered, washed with CH_2Cl_2, dried, and hydrolyzed in sealed ignition tubes containing HCl–propionic acid (1:1) for 6 h at 130 °C.[14] The analyses are given in Table I (runs 1–3).

B. Reaction with TFA–HBr–HOAc and HF. Untreated Boc-Lys(Z)-resin (0.100 g) was cleaved with TFA–32% HBr in HOAc (1:1) (1 h) and worked up as described for the TFA–HBr–HOAc cleavage of Boc-Gly-Lys(Z)-resin. Untreated Boc-Lys(Z)-resin (0.100 g) was also cleaved with anhydrous HF, in the absence of anisole, for 1 h at room temperature. The HF was removed in vacuo and the resin was

extracted with 1% HOAc. The use of either TFA–HBr–HOAc or HF did not allow formation (<0.1%) of N^{ϵ}-Bzl-Lys (runs 4 and 5, Table I).

C. Reaction with Cleavage Reagents Containing Carbonium Ion Scavengers. Solutions (2–4 ml) having the compositions listed in Table II were shaken with samples of Boc-Lys(Z)-resin (0.100–0.200 g) for 14 h at room temperature. The resins were filtered, washed with CH_2Cl_2, and cleaved with TFA–32% HBr in HOAc (1:1) as described above. The results are given in Table II.

Deprotection of Z-Gly and Lys(Z) in Refluxing Trifluoroacetic Acid. Solutions of Z-Gly (0.01, 0.10, 1.0 M) and Lys(Z) (0.1 M) in anhydrous TFA were prepared. Duplicate solutions containing 20% anisole were also prepared. The solutions were refluxed for 30 min and then evaporated in vacuo. The resulting residues were taken up in water and extracted twice with CH_2Cl_2. The aqueous solutions were analyzed for amino acid and *N*-benzylamino acid. Recoveries of 95–101% were observed. The results are given in Table III.

Deprotection of Z-Lys in Refluxing Trifluoroacetic Acid. A solution of Z-Lys[33] (1.0 M) was refluxed (30 min) in TFA and worked up as described above. No N^{ϵ}-Bzl-Lys (<0.1%) was detected. The presence of N^{α}-Bzl-Lys (0.6%) was established when a reference sample of N^{α}-Bzl-Lys, prepared by treating Bzl-Lys(Z)[37] with 32% HBr in HOAc, was chromatographed on the short column of the analyzer.

Deprotection of Z-Gly and Lys(Z) in Trifluoromethanesulfonic Acid–Trifluoroacetic Acid. Trifluoromethanesulfonic acid (8.3 equiv) was added to a solution of Z-Gly (0.1 M) in TFA containing anisole (2.3 equiv) with the immediate formation of a purple solution. An aliquot was removed at 30 min, quenched in pH 4.25 buffer, and analyzed. It contained N^{α}-Bzl-Gly (1.2%) and Gly (98.8%).

The experiment was repeated using Lys(Z) (0.1 M) in TFA containing anisole (2.6 equiv) and trifluoromethanesulfonic acid (9.4 equiv). At 30 min an aliquot was removed, quenched in pH 7.00 buffer, and analyzed. It contained N^{ϵ}-Bzl-Lys (3.1%) and Lys (96.9%).

Deprotection of Z-Gly and Lys(Z) in HF. Z-Gly (20.9 mg, 100 μmol) and Lys(Z) (28.0 mg, 100 μmol) were allowed to stir in anhydrous HF (5 ml) for 30 min at room temperature. The HF was removed in vacuo and the resultant residue was extracted with water. An insoluble, light yellow solid was filtered off and the filtrate was evaporated in vacuo, yielding a residue which was dissolved in water (12.5 ml) for ion-exchange chromatography. Neither N^{α}-Bzl-Gly nor N^{ϵ}-Bzl-Lys could be detected (<0.1%).

Deprotection of Lys(2,4-Cl_2Z) in HF. Lys(2,4-Cl_2Z) (70.4 mg, 202 μmol) was stirred in anhydrous HF (10 ml) for 1 h at room temperature. The HF was removed in vacuo and the resulting material was extracted with pyridine acetate buffer (0.8 M, pH 5.2). A white solid was removed by filtration and a portion of the filtrate was subjected to ion-exchange chromatography. Less than 0.1% Lys(2,4-Cl_2Bzl) was present.

Registry No.—N^{α}-Bzl-Gly·HCl, 7689-50-1; N^{ϵ}-Bzl-Lys·HCl, 38299-38-6; N^{α}-Z-N^{ϵ}-(2,4-Cl_2Bzl)-Lys, 58581-65-0; *t*-Bu-Gly-Lys(Z), 58581-66-1; N^{ϵ}-benzylidene-L-lysine, 14511-39-8; 2,4-dichlorobenzaldehyde, 874-42-0; N^{α}-carbobenzoxy-L-lysine, 2212-75-1; N^{α}-chloroacetyl-N^{ϵ}-benzyloxycarbonyl-L-lysine, 47376-73-8; *tert*-butylamine, 75-64-9; Z-Gly, 1138-80-3; N^{ϵ}-Z-Lys, 1155-64-2; Boc-Lys(Z)-H, 2389-60-8; Lys(2,4-Cl_2Z), 58581-67-2.

References and Notes

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(2) M. Bergmann and L. Zervas, *Ber. Dtsch. Chem. Ges.*, **65**, 1192 (1932).
(3) L. A. Carpino, *J. Am. Chem. Soc.*, **79**, 98, 4427 (1957); F. C. McKay and N. F. Albertson, *ibid.*, **79**, 4686 (1957); G. W. Anderson and A. C. McGregor, *ibid.*, **79**, 6180 (1957).
(4) P. Sieber and B. Iselin, *Helv. Chim. Acta*, **51**, 614 (1968).
(5) P. Sieber and B. Iselin, *Helv. Chim. Acta*, **51**, 622 (1968).
(6) T. Sheradsky, G. Salemnick, and M. Frankel, *Isr. J. Chem.*, **9**, 263 (1971).
(7) R. S. Hodges and R. B. Merrifield, *Anal. Biochem.*, **65**, 241 (1975).
(8) L. C. Dorman, *Tetrahedron Lett.*, 2319 (1969).
(9) B. F. Gisin, *Anal. Chim. Acta*, **58**, 278 (1972).
(10) K. R. Rao, S. M. Birnbaum, R. B. Kingsley, and J. P. Greenstein, *J. Biol. Chem.*, **198**, 507 (1952).
(11) F. Weygand and W. Steglich, *Z. Naturforsch. B*, **14**, 472 (1959).
(12) J. M. Manning and S. Moore, *J. Biol. Chem.*, **243**, 5591 (1968).
(13) R. B. Merrifield, A. R. Mitchell, and J. E. Clarke, *J. Org. Chem.*, **39**, 660 (1974).
(14) J. Scotchler, R. Lozier, and A. B. Robinson, *J. Org. Chem.*, **35**, 315 (1970).
(15) M. Friedman and L. H. Krull, *Biochim. Biophys. Acta*, **207**, 361 (1970).
(16) L. Benoiton, *Can. J. Chem.*, **42**, 2043 (1964).
(17) B. F. Gisin, unpublished procedure. *Caution.* Pressure develops.
(18) S. Sakakibara, Y. Shimonishi, Y. Kishida, M. Okada, and H. Sugihara, *Bull. Chem. Soc. Jpn.*, **40**, 2164 (1967).
(19) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963).
(20) B. W. Erickson and C. Y. Wang, Abstracts, 170th National Meeting of the American Chemical Society, Chicago, Ill., August 1975, No. ORGN-81.
(21) S. Sakakibara, T. Fukuda, Y. Kishida, and I. Honda, *Bull. Chem. Soc. Jpn.*, **43**, 3322 (1970).
(22) D. Yamashiro and C. H. Li, *J. Am. Chem. Soc.*, **95**, 1310 (1973).
(23) B. W. Erickson and R. B. Merrifield, *J. Am. Chem. Soc.*, **95**, 3757 (1973).
(24) Some examples of decarbobenzoxylations using trifluoroacetic acid include S. S. Wang and R. B. Merrifield, *Int. J. Pept. Protein Res.*, **4**, 309 (1972); W. König and R. Geiger, *Chem. Ber.*, **105**, 2872 (1972); W. Voelter, S. Fuchs, and K. Zech, *Tetrahedron Lett.*, 3975 (1974); M. Bodanszky, Y. S. Klausner, C. Y. Lin, V. Mutt, and S. Said, *J. Am. Chem. Soc.*, **96**, 4973 (1974).
(25) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).
(26) H. Yajima, N. Fujii, H. Ogawa, and H. Kawatani, *J. Chem. Soc., Chem. Commun.*, 106 (1974).
(27) J. Pless and W. Bauer, *Angew. Chem., Int. Ed. Engl.*, **12**, 147 (1973).
(28) F. C. McKay and N. F. Albertson, *J. Am. Chem. Soc.*, **79**, 4686 (1957); F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 1 (1962).
(29) B. E. B. Sandberg and U. Ragnarsson, *Int. J. Pept. Protein Res.*, **6**, 111 (1974).
(30) B. F. Gisin and R. B. Merrifield, *J. Am. Chem. Soc.*, **94**, 3102 (1972).
(31) C. V. Greco, W. H. Nyberg, and C. C. Cheng, *J. Med. Chem.*, **5**, 861 (1962).
(32) H. J. Haas, *Chem. Ber.*, **94**, 2442 (1961).
(33) B. Bezas and L. Zervas, *J. Am. Chem. Soc.*, **83**, 719 (1961).
(34) The mass spectroscopy was performed by Dr. M. E. Rennekamp in the laboratory of Professor F. H. Field (Mass Spectrometric Biotechnology Resource, Rockefeller University).
(35) This derivative was prepared by Dr. R. S. Hodges.
(36) B. F. Gisin, *Helv. Chim. Acta*, **56**, 1476 (1973).
(37) P. Quitt, J. Hellerbach, and K. Vogler, *Helv. Chim. Acta*, **46**, 327 (1963).