Solid-Phase Synthesis of Protected Peptide Hydrazides. Preparation and Application of Hydroxymethyl Resin and 3-(*p*-Benzyloxyphenyl)-1,1-dimethylpropyloxycarbonylhydrazide Resin

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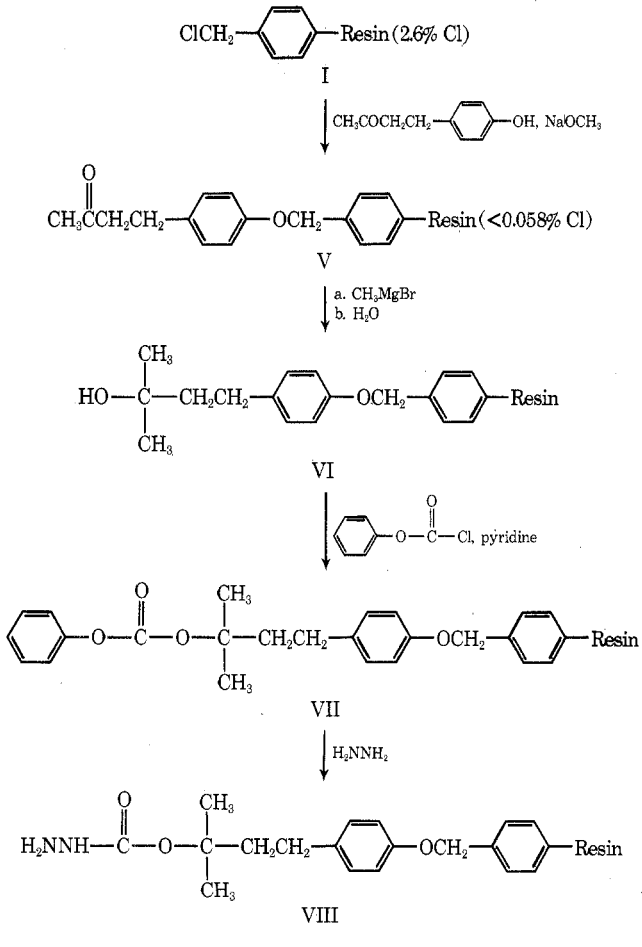
Convenient procedures for the preparation of hydroxymethyl resin (III) and 3-(*p*-benzyloxyphenyl)-1,1-dimethylpropyloxycarbonylhydrazide resin (VIII) [$H_2NNHCOOC(CH_3)_2CH_2CH_2C_6H_4OCH_2C_6H_4$ resin] were developed. Reaction of potassium acetate with Merrifield resin gave acetoxymethyl resin, which on reduction or hydrazinolysis produced III. For the preparation of VIII, Merrifield resin was treated with 4-(*p*-hydroxyphenyl)-2-butanone to give the ketone resin, which was treated with CH_3MgBr giving rise to tertiary alcohol resin. On reaction with phenyl chloroformate, followed by hydrazinolysis, VIII was obtained. Resin III was applied to the synthesis of Aoc-Arg(Tos)-Val-Tyr(Cl_2Bzl)-$HNNH_2$ and Boc-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-$HNNH_2$ (IX) by hydrazinolysis of peptide to resin ester bond. Using the synthesis of IX as a model system, a comparative study was made of the following coupling methods: oxidation–reduction condensation, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline method; dicyclohexylcarbodiimide; dicyclohexylcarbodiimide plus *N*-hydroxybenzotriazole; and diphenylphosphoryl azide. Resin VIII was applied to the synthesis of Fmoc-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-$HNNH_2$, Fmoc-Gly-Ala-Val-Leu-$HNNH_2$, and Z(2-NO_2)-Gly-Val-Ala-Leu-$HNNH_2$. These peptide hydrazides were obtained by cleavage of the anchoring bond with 50% TFA in CH_2Cl_2.

Solid-phase syntheses of protected peptide hydrazides either by hydrazinolysis of peptide chains attached to Merrifield resin[1-7] or by the application of "hydrazide resins"[8-10] has been described in the literature. These procedures can provide convenient intermediates for polypeptide synthesis via fragment condensation, allowing the combination of solid-phase techniques[11-16] and classical procedures[16-18] and retaining the best features of each method. In this report, the synthesis of protected peptide hydrazides by the use of the hydroxymethyl resin[13,19] and 3-(*p*-benzyloxyphenyl)-1,1-dimethylpropyloxycarbonylhydrazide resin is described.

The hydroxymethyl resin (III) was prepared from Merrifield resin (I) (chloromethylated copolystyrene–1% divinylbenzene, 2.6% Cl) by stirring in dimethylacetamide with slight excess of potassium acetate to form acetoxymethyl resin II, which on reduction with $LiAlH_4$ or hydrazinolysis yielded the desired hydroxymethyl resin III. These reactions were conveniently monitored either by ir spectrophotometry or microanalysis and were found to proceed smoothly. The hydroxymethyl resin (III) was then esterified with *tert*-butyloxycarbonylamino acids by the dicyclohexylcarbodiimide method[20] utilizing pyridine[10,21] or 4-dimethylaminopyridine[22,23] as catalysts. The Boc-aminoacyl resins[24] thus prepared have the degree of substitution generally in the range of 0.5–0.6 mmol/g. In order to prevent unreacted excess hydroxymethyl groups present on the resin from interfering with subsequent reactions, Boc-aminoacyl resins were benzoylated as reported previously.[10] This procedure for the preparation and use of the hydroxymethyl resin is more reproducible and adaptable to larger scale preparation than those described in the literature.[13,25,26] The advantages of using hydroxymethyl resin rather than chloromethyl resin have already been discussed.[12,25,26]

The synthesis of 3-(*p*-benzyloxyphenyl)-1,1-dimethylpropyloxycarbonylhydrazide resin (VIII) is depicted in Scheme I. The starting material I (0.73 mmol/g) was treated with 4-(*p*-hydroxyphenyl)-2-butanone in the presence of an equivalent amount of $NaOCH_3$. The resulting "ketone" resin (V) absorbed strongly at 1710 cm^{-1} and contained less than 0.058% Cl (0.016 mmol/g). Treatment of V with freshly prepared CH_3MgBr followed by hydrolysis of the ensuing product afforded the "tertiary alcohol resin"

Scheme I
Preparation of 3-(*p*-Benzyloxyphenyl)-1,1-dimethylpropyloxycarbonylhydrazide Resin



(VI). As is evident from the ir spectrum, the carbonyl function had disappeared completely while the alkyl aryl ether band at 1230 cm^{-1} remained practically unchanged. The tertiary alcohol group of VI was then converted into a mixed carbonate (VII) which on hydrazinolysis gave rise to

Table I
Synthesis of Boc-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-$HNNH_2$ Using Different Coupling Methods

Coupling agents[a]	Mp, °C	$[\alpha]^{25}$D, deg	Yield, %	Anal., %		
				Calcd	67.30	6.63	10.56
					C	H	N
DCC[b]	227–229	−2.64	61.5		67.00	6.68	10.43
DCC + HOBT[c]	228–230	−1.18	57.5		66.72	6.65	10.54
EEDQ[c]	229–230	−1.29	55.8		66.73	6.53	10.56
TPP + DPDS[d]	229–230	−1.91	57.7		67.53	6.80	10.50

[a] DCC, dicyclohexylcarbodiimide; HOBT, *N*-hydroxybezotriazole; EEDQ, *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; TPP, triphenylphosphine; DPDS, 2,2′-dipyridyl disulfide. [b] Coupling time, 2 hr (reaction progress with time not monitored). [c] Coupling time, 22 hr. [d] Coupling time, 3 hr.

the desired product VIII containing 2.0% of nitrogen (0.71 mmol/g of H_2NNH_2). The ir spectrum showed the expected changes in the wavelength of the carbonyl absorption. All reactions appeared to have proceeded completely.

The hydroxymethyl resin III was used as solid support for the synthesis of Boc-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-$HNNH_2$ (IX). Boc-Thr(Bzl)-OCH_2-C_6H_4-Resin was deprotected (50% TFA) and neutralized (10% TEA) and the solid-phase synthesis was continued with sequential incorporation of Boc-Tyr(Bzl)-OH, Boc-Phe-OH, Boc-Phe-OH, and Boc-Gly-OH into the growing peptide chain on the resin according to the general Merrifield technique.[11-14] During the synthesis a 2.6-fold excess of both Boc-amino acid and DCC[20] was used in each of the coupling reactions. The product Boc-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-OCH_2-C_6H_4-Resin thus obtained was treated with hydrazine in DMF to give analytically pure crystalline IX in 61.5% overall yield. The synthesis of IX was repeated under exactly the same conditions except that different coupling procedures[20,27-32] were used for evaluation in solid-phase synthesis. The results are summarized in Table I. In agreement with recent reports by Mukaiyama et al.,[29,30] it was evident that the oxidation–reduction procedure is well suited for solid-phase peptide synthesis. The method, moreover, has been claimed to be free of complications when applied to the synthesis of asparagine or glutamine containing peptides. This aspect of the oxidation–reduction procedure was further studied by the synthesis of pGlu-Gln-Ala-NH_2, and indeed the synthesis was found to proceed satisfactorily. The tripeptide amide identical with that prepared previously[33] with the *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline procedure[31] was obtained in 79% overall yield. There were no indications of nitrile formation during the synthesis as judged by ir and NMR spectrophotometry.

The hydroxymethyl resin III was also utilized for the synthesis of L-pyroglutamyl-L-serylglycinamide (X). Boc-Gly-OCH_2-C_6H_4-Resin was deprotected and neutralized as usual[11,13] and the solid-phase synthesis was carried out by coupling Boc-Ser(Bzl)-OH to the resin followed by pyroglutamic acid. The tripeptide resin thus obtained was then stirred with ammonia–saturated methanol, giving the crystalline O-protected tripeptide amide pGlu-Ser(Bzl)-Gly-NH_2 in 38% overall yield. This compound gave X upon hydrogenation. A peptide isolated from hypothalamic extracts has been reported to possess this structure and to exhibit pituitary growth hormone releasing activity.[34] However, synthetic pGlu-Ser-Gly-NH_2 (X) prepared in several laboratories, including our own,[35,36] did not elicit such activity in several systems thus far tested.

Another protected peptide hydrazide, Aoc-Arg(Tos)-Val-Tyr(Cl_2Bzl)-$HNNH_2$ (XI), was also prepared on the hydroxymethyl resin III in an analogous manner. The tripeptide resin was hydrazinolyzed in methanolic hydrazine

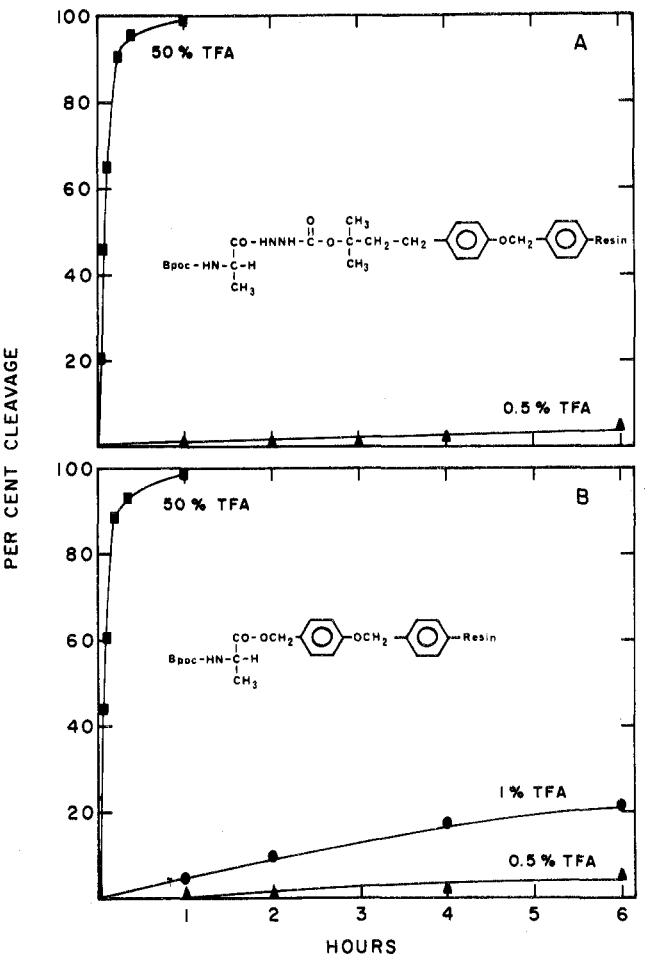


Figure 1. The rate of cleavage of the anchoring bond on Bpoc-Ala derivatives of 3-(*p*-benzyloxyphenyl)-1,1-dimethylpropyloxycarbonylhydrazide resin (A), and *p*-alkoxybenzyl alcohol resin (B) in various concentrations of trifluoroacetic acid in CH_2Cl_2. Data shown in these figures were derived from the nitrogen analyses on the corresponding aminoacyl resins.

solution and the pure crystalline product XI was obtained in 64% overall yield.

Peptide hydrazides prepared with the use of hydroxymethyl resin crystallized more readily than the products obtained by hydrazinolysis of standard chloromethyl group containing peptide resins, which are contaminated with hydrazine hydrochloride.

The 3-(*p*-benzyloxyphenyl)-1,1-dimethylpropyloxycarbonylhydrazide resin VIII was utilized to prepare Z(2-NO_2)-Gly-Val-Ala-Leu-$HNNH_2$ (XII). The photosensitive Z(2-NO_2) group[37,38] was used for amino terminal protection. The product was obtained in 44.4% overall yield as calculated from the hydrazide content of resin VIII. The solid-phase synthesis was conducted using 2.5-fold excesses

each of the respective amino acid derivatives and DCC for coupling in each cycle under previously detailed conditions.[8-10] The Bpoc-amino protecting group was removed by 10-min treatment with 0.5% TFA in CH_2Cl_2.[8] Two protected peptide hydrazides containing the base-labile Fmoc amine protecting group[39] were also prepared. Fmoc-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-$HNNH_2$ (XIII) was obtained in 36% and Fmoc-Gly-Ala-Val-Leu-$HNNH_2$ (XIV) in 49% overall yield. The combined use of resin VIII with Bpoc-amino acids together with amino terminal Z(2-NO_2) or Fmoc protection provides a potential for ready preparation of a large variety of peptide intermediates which contain protecting groups suitable for polypeptide synthesis via the fragment condensation strategy. A *tert*-alkoxycarbonylhydrazide resin support[8,9] that can be used interchangeably with VIII has been described previously but its preparation required the use of the hazardous liquid HF. The procedure described in this report for the preparation of VIII appears to be simpler and more practical.

The relative stabilities of the anchoring bonds on aminoacyl resin VIII in different concentrations of TFA were studied. Bpoc-Ala-HNNH-COO-$C(CH_3)_2CH_2CH_2C_6H_6O$-$CH_2C_6H_4$-Resin was stirred with 20 volumes of 0.5 or 50% TFA in CH_2Cl_2. At various time intervals, samples were withdrawn and the resin was collected, washed, and examined by ir spectrophotometry as well as by nitrogen analysis. As the anchoring bond was cleaved, both the intense broad band at 1680–1700 cm^{-1} in the ir spectrum and the nitrogen content of the resin decreased progressively (Figure 1A). For comparison, a similar set of experiments was performed on Bpoc-Ala-$OCH_2C_6H_4OCH_2C_6H_4$-Resin[10] (Figure 1B). As expected, the anchoring bonds on both resins were stable to the conditions of Bpoc deprotection (0.5% trifluoroacetic acid, 10 min) but were rapidly cleaved by 50% trifluoroacetic acid within a few minutes.

Experimental Section

Melting points are uncorrected. The infrared spectra were taken on a Perkin-Elmer Model 137 spectrophotometer using KBr pellets. Thin layer chromatography was carried out on precoated silica gel plates (Merck, F-254) with the following solvent systems: 1-butanol–acetic acid–water (4:1:1), 1-butanol–pyridine–acetic acid–water (15:10:3:12); 1-butanol–ethyl acetate–acetic acid–water (1:1:1:1); 1-propanol–water (7:3). Microanalyses and other physicochemical measurements were carried out by the Physical Chemistry Department.

Merrifield resin (chloromethylated copolystyrene–1% divinylbenzene, 200–400 mesh, 2.6% Cl) used in these experiments was purchased from Bio-Rad Laboratories. Amino acid derivatives were obtained from Bachem, Inc., Marina Del Ray, Calif., or Chemical Dynamics Corp., South Plainfield, N.J. All Bpoc-, Z(2-NO_2)-, and Fmoc-amino acid derivatives were prepared in this laboratory according to literature procedures.[38-42] All optically active amino acids used were of the L configuration.

Hydroxymethyl Polystyrene–1% Divinylbenzene Resin (III). Merrifield resin (I, 71 g, 52 mmol) was suspended in dimethylacetamide (600 ml) and stirred gently with potassium acetate (6.26 g, 64 mmol) at 85° for 24 hr. The acetoxymethyl resin (II) thus formed was washed with DMF, dioxane, and methanol to yield 77.4 g of buff-colored material: ir (KBr) 1720 cm^{-1}; Cl <0.12% (0.034 mmol/g). II was suspended in anhydrous ether (1.8 l.) and treated with $LiAlH_4$ (7.0 g) added in small portions during 20 min. After 4 hr of additional stirring, the resin was transferred to a glass filter and washed with ethyl acetate, dioxane, and methanol. The slightly grayish color was removed by stirring in a 1:2 mixture of 1 *N* H_2SO_4–dioxane (4.5 l.) for 24 hr. This operation was repeated once more and the snow-white hydroxymethyl resin thus obtained weighed 72.2 g. The carbonyl band in the ir spectrum disappeared completely.

Conversion of II (23.5 g) into III (23.0 g) was also accomplished by stirring in 250 ml of DMF containing 25 ml of anhydrous H_2NNH_2 for 76 hr. The ir spectrum of the product III was identical with that of the hydroxymethyl resin prepared by the $LiAlH_4$ procedure described above.

Esterification of Hydroxymethyl Resin III with Boc-Amino Acids. III (5 g, 3.65 mmol) was suspended in CH_2Cl_2 (50 ml) and treated with pyridine (1.5 ml), Boc-Ala-OH (3 g, 16 mmol), and DCC (3.5 g, 17 mmol) for 120 min. The product was washed with CH_2Cl_2, DMF, and methanol to yield colorless Boc-Ala-OCH_2-C_6H_4-Resin (5.33 g): ir 1720 and 1735 cm^{-1}; N, 0.88% (0.63 mmol/g); alanine, 0.62 mmol/g. The resin was further treated with pyridine (1.65 ml) and benzoyl chloride (1.95 ml) in CH_2Cl_2 (50 ml) at 0° for 15 min. The ensuing product (5.35 g) showed a slight increase in ir absorption at 1720 cm^{-1}.

For certain Boc-amino acids, esterification to III requires a more powerful catalyst. Thus, III was suspended in CH_2Cl_2–DMF mixture (20 g/200 ml) and stirred with 4-dimethylaminopyridine (3.66 g, 30 mmol), Boc-Gly-OH (5.25 g, 30 mmol), and DCC (6.6 g, 32 mmol) for 120 min. The esterified resin (20.5 g) absorbed strongly at 1720 and 1730 cm^{-1}, N, 0.90% (0.64 mmol/g). The resin was benzoylated in an analogous manner as above to give the desired Boc-Gly-OCH_2-C_6H_4-Resin (20.6 g).

Similarly prepared were resin III esters of Boc-Phe-OH, Boc-Tyr(Cl_2Bzl)-OH, Boc-Thr(Bzl)-OH, Boc-Pro-OH, Boc-β-Ala-OH, and Z-Lys(BOC)-OH.

3-(*p*-Benzyloxyphenyl)-1,1-dimethylpropyloxycarbonylhydrazide Resin (VIII). Merrifield resin (10 g, 7.3 mmol) was suspended in DMF (70 ml) and treated with 4-(*p*-hydroxyphenyl)-2-butanone (1.64 g, 10 mmol) in the presence of $NaOCH_3$ (0.54 g, 10 mmol) at 85° for 24 hr. The dark brownish reaction mixture was filtered and the resin was washed with DMF, CH_2Cl_2, and MeOH to give 10.9 g of light buff colored material: ir 1730 cm^{-1}; Cl, 0.058%. The resin was then suspended in benzene (200 ml) and treated with a Grignard reagent (CH_3MgBr) freshly prepared from 0.54 g of Mg turnings in ether (300 ml) bubbled with dry CH_3Br. After 60 min of additional stirring, the resin was washed (benzene, dioxane) and stirred in a mixture of 1 *N* H_2SO_4–dioxane (1:2) for 120 min. The tertiary alcohol resin (VI) thus formed was collected and washed with H_2O–dioxane (1:1), dioxane, DMF, and CH_2Cl_2 and then treated with pyridine (7.9 ml) and phenyl chloroformate (9.8 ml) in 120 ml of CH_2Cl_2 at 0° for 16 hr. The reaction mixture was poured into ice–water (100 ml) and filtered to collect the resin. The mixed carbonate resin (VII) thus obtained was washed with more ice–water, dioxane, and DMF. It was then stirred with 120 ml of DMF containing 10 ml of anhydrous H_2NNH_2 for 6 hr to give the desired product VIII: N, 2.00% (0.71 mmol/g).

2-Nitrobenzyl-*p*-nitrophenyl Carbonate. 2-Nitrobenzyl alcohol (25 g, 163 mmol) was dissolved in CH_2Cl_2 (300 ml) and allowed to react with pyridine (21.5 ml) and *p*-nitrophenyl chloroformate (34.2 g, 170 mmol) overnight at 0°. The mixture was mixed with ice–water (500 ml) and diluted with CH_2Cl_2 (300 ml) in a separatory funnel. The organic layer was then washed with 0.02 *N* HCl followed by water. After drying over Na_2SO_4, the solvent was removed by evaporation at 35° and the remaining solid was recrystallized from 200 ml ethyl acetate: yield 41.8 g (91%); mp 133–136°.

Anal. Calcd for $C_{14}H_{10}N_2O_7$ (318.24): C, 52.84; H, 3.17; N, 8.80. Found: C, 53.12; H, 3.40; H, 8.78.

2-Nitrobenzyloxycarbonylglycine. Glycine (1.5 g, 2 mmol) was mixed with 9.5 ml of Triton B (40% methanolic solution of benzyltrimethylammonium hydroxide) and evaporated to dryness at 35°. The residue was evaporated twice with 18 ml each of DMF and stirred with 7.0 g of 2-nitrobenzyl-*p*-nitrophenyl carbonate (2.2 mmol) at 40° for 120 min. The reaction mixture was partitioned between ethyl acetate and water (300 ml each) and the aqueous layer was washed twice with ethyl acetate and acidified to pH 2.5 with 1 *M* citric acid. The resulting oily product was extracted into ethyl acetate, washed with water, dried over Na_2SO_4, and evaporated at 35°, leaving a heavy syrup. It was taken up in a small volume of ethyl acetate and treated with petroleum ether. The ensuing crystalline solid was recrystallized from the same solvents: yield 3.35 g (66%); mp 121–123°.

Anal. Calcd for $C_{10}H_{10}N_2O_6$ (254.2): C, 47.25; H, 3.97; N, 11.02. Found: C, 47.17; H, 4.18; N, 10.99.

This compound has been listed in a communication by Patchornik et al.[38] (mp 120–122°) without details of the synthesis.

Boc-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-$HNNH_2$ (IX). Boc-Thr(Bzl)-OCH_2-C_6H_4-Resin (1.4 g, 0.77 mmol) was placed in a reaction vessel[43] on a shaker and the solid-phase peptide synthesis conducted by sequential incorporation of Boc-Tyr(Bzl)-OH (0.62 g, 2.0 mmol), Boc-Phe-OH (0.53 g, 2.0 mmol), Boc-Phe-OH (0.53 g, 2.0 mmol), and Boc-Gly-OH (0.35 g, 2.0 mmol) according to the general principles of the Merrifield technique.[11] The protected pentapeptide resin (1.8 g) thus obtained was suspended in DMF (20 ml) and stirred gently with anhydrous hydrazine (2 ml) for 66

hr. The peptide hydrazide was separated from the resin by filtration and evaporated at 35° to a syrup which upon treatment with ether solidified immediately. The solid was then triturated in hot methanol (0.54 g, mp 224–227°) and crystallized from DMF (15 ml) by slow addition of ethanol (30 ml): yield 0.44 g (61.5%); mp 227–229°; $[\alpha]^{25}D$ −2.64° (*c* 1, DMF); NMR spectral data agreed with the expected values.

Anal. Calcd for $C_{52}H_{61}N_7O_9$ (928.07): C, 67.30; H, 6.63; N, 10.56. Found: C, 67.00; H, 6.68; N, 10.43.

The synthesis of IX was repeated under exactly the same conditions except that different coupling methods were employed in each experiment. The results of these experiments are summarized in Table I. Thus, 1 equiv of DCC and 2 equiv of *N*-hydroxybenzotriazole relative to Boc-amino acid were employed with reaction time of 22 hr. The experiment using *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline was conducted with equivalent amounts of coupling agent and Boc-amino acid with a reaction time of 22 hr. For the experiment using the oxidation–reduction method, equivalent amounts of 2,2′-dipyridyl disulfide, triphenylphosphine, and Boc-amino acid were used and the coupling time was 3 hr. As in the experiment using DCC as coupling agent, a 2.6-fold excess of Boc-amino acid derivatives relative to amino groups on the peptide resin was used throughout all of these experiments. Experiments with diphenylphosphoryl azide[32] in solid-phase synthesis (22 hr coupling time) under similar conditions were unsuccessful.

Aoc-Arg(Tos)-Val-Tyr(Cl_2Bzl)-$HNNH_2$ (XI). Hydroxymethyl resin (III, 9.4 g, 6.9 mmol) was stirred in CH_2Cl_2 (95 ml) with 4-dimethylaminopyridine (1.72 g, 14 mmol), Boc-Tyr(Cl_2Bzl)-OH (6.2 g, 14 mmol),[44] and DCC (3.3 g, 16 mmol) for 45 min and the esterified resin (11.8 g) was collected and benzoylated as described above to give 11.4 g of Boc-Tyr(Cl_2Bzl)-OCH_2-C_6H_4-Resin: N, 0.88% (0.63 mmol); Cl, 4.23% (0.6 mmol amino acid/g). Part of this material (6 g, 3.72 mmol) was used in the subsequent solid-phase synthesis using Boc-Val-OH (2.02 g, 9.3 mmol) and Aoc-Arg(Tos)-OH (3.41 g, 9.3 mmol) in each cycle. The tripeptide resin Aoc-Arg(Tos)-Val-Tyr(Cl_2Bzl)-OCH_2-C_6H_4-Resin (7.8 g) was hydrazinolyzed in 300 ml of MeOH containing 30 ml of anhydrous H_2NNH_2 for 72 hr (25°). The resin was filtered off and the filtrate was evaporated to a glassy solid (3.2 g). It was crystallized from *i*-PrOH and then recrystallized from EtOH: yield 2.1 g (64%); mp 189–191°; $[\alpha]^{25}D$ −13.66° (*c* 1, DMSO); NMR spectral data agreed with the structure.

Anal. Calcd for $C_{40}H_{54}N_8O_8SCl_2$ (877.89): C, 54.73; H, 6.20; N, 12.76; S, 3.65; Cl, 8.08. Found: C, 54.47; H, 6.39; N, 12.60; S, 3.70; Cl, 8.20.

pGlu-Ser(Bzl)-Gly-NH_2. Boc-Gly-OCH_2-C_6H_4-Resin (9.0 g, 5.76 mmol) was placed in a 200-ml peptide synthesis flask[43] and the solid-phase synthesis carried out as described above with Boc-Ser(Bzl)-OH (5.1 g, 17.3 mmol) and pyroglutamic acid (2.24 g, 17.3 mmol) sequenally incorporated into the resin using DCC (3.57 g, 17.4 mmol) as coupling agent in each cycle. The tripeptide resin (10.2 g) was suspended in dry methanol (500 ml), bubbled with dry NH_3 at 0° until nearly saturated, and stirred for 66 hr. The tripeptide amide liberated from the resin was worked up as usual, giving rise to a syrup which upon treatment with ethyl acetate solidified immediately. The crude material was dissolved in methanol (50 ml) and crystallized by slow addition of ether: yield 1.21 g (58%); mp 143–145°; $[\alpha]^{25}D$ +5.25° (*c* 0.9, MeOH); NMR spectral data agreed with the expected values.

Anal. Calcd for $C_{17}H_{22}N_4O_5$ (362.38): C, 56.35; H, 6.12; N, 15.46. Found: C, 56.26; H, 6.16; N, 15.56.

The same compound was synthesized again using the oxidation–reduction method[29,30] with a 2.0-fold excess each of amino acid derivative, 2,2′-dipyridyl disulfide, and triphenylphosphine, and a 3-hr coupling time. From 6.63 g of Boc-Gly-$OCH_2C_6H_4$-Resin (4.2 mmol) the desired product pGlu-Ser(Bzl)-Gly-NH_2 (0.85 g) was obtained in 56% overall yield: mp 143–145°; $[\alpha]^{25}D$ +6.37° (*c* 1, MeOH).

Anal. Found: C, 56.57; H, 6.10; N, 15.47.

pGlu-Ser-Gly-NH_2 (X). The above compound (0.5 g, 1.38 mmol) was dissolved in a solvent mixture (50 ml of methanol, 15 ml of water, 0.5 ml of acetic acid) and hydrogenated at 55 psi in a Parr hydrogenator overnight in the presence of 0.2 g of catalyst (5% Pd on $BaSO_4$). The catalyst was filtered off and the filtrate was evaporated to an oil which was taken up in 35 ml of water and lyophilized to give a hygroscopic white powder (0.53 g). It was dissolved in 15 ml of methanol, filtered to remove small insolubles, and treated with a 2:1 mixture of ether and tetrahydrofuran until turbid. The product crystallized slowly when stored in the refrigerator: yield 0.228 g (59.7%); mp 168–172°; $[\alpha]^{25}D$ −10.76° (*c* 0.7, MeOH); NMR spectral data agreed with the expected values.

Anal. Calcd for $C_{10}H_{16}N_4O_5 \cdot \frac{1}{4}H_2O$ (276.76): C, 43.33; H, 5.98; N, 20.17. Found: C, 43.36; H, 5.91; N, 20.22.

pGlu-Gln-Ala-NH_2. The solid-phase synthesis was carried out as usual on Boc-Ala-OCH_2-C_6H_4-Resin (1.42 g, 0.88 mmol) using a 2.2-fold excess each of Boc-Gln-OH (0.845 g), 2,2′-dipyridyl disulfide (0.49 g), and triphenylphosphine (0.58 g) in the first cycle and pyroglutamic acid (0.284 g) with the same amount of coupling agents in the second cycle. The reaction time was set at 3 hr. The tripeptide resin (1.6 g) obtained was ammonolyzed and worked up as usual to give 0.227 g (79%) of pGlu-Gln-Ala-NH_2 identical with the same compound prepared[33] by the *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline method: mp 260–262°; ir spectrum and NMR spectrum identical with the reference spectra.

Z(2-NO_2)-Gly-Val-Ala-Leu-$HNNH_2$ (XII). The hydrazide resin VIII (1.4 g, 0.98 mmol) was placed in a reaction vessel[43] and the solid-phase synthesis carried out by incorporating Bpoc-Leu-OH (0.94 g, 2.55 mmol), Bpoc-Ala-OH (0.84 g, 2.55 mmol), Bpoc-Val-OH (0.91 g, 2.55 mmol), and Z(2-NO_2)-Gly-OH (0.64 g, 2.55 mmol) successively into the resin according to the procedure described previously.[8–10] The tetrapeptide hydrazide resin thus obtained (1.86 g) was stirred in CH_2Cl_2 (18 ml) for a few minutes and an equal volume of TFA was added. After 30 min the resin was filtered off and the filtrate evaporated at 30° to a syrup. It was evaporated twice more with fresh CH_2Cl_2 and treated with ethyl acetate (100 ml). The white solid obtained was triturated in hot methanol (10 ml) and crystallized from DMF by the addition of ether: yield 0.24 g (44.4%); mp 224–228°; $[\alpha]^{25}D$ −16.20° (*c* 1, DMF); NMR spectral data agreed with the expected values.

Anal. Calcd for $C_{24}H_{37}N_7O_8$ (551.6): C, 52.26; H, 6.76; N, 17.78. Found: C, 52.30; H, 6.84; N, 17.63.

Fmoc-Gly-Phe-Phe-Tyr(Bzl)-Thr(Bzl)-$HNNH_2$ (XIII). The hydrazide resin VIII (1.4 g, 1.0 mmol) was placed in the peptide synthesis flask and the synthesis conducted as usual[8–10] with Bpoc-Thr(Bzl)-OH (1.12 g, 2.5 mmol), Bpoc-Tyr(Bzl)-OH (1.27 g, 2.5 mmol), Bpoc-Phe-OH (1.01 g, 2.5 mmol), Bpoc-Phe-OH (1.01 g), and Fmoc-Gly-OH (0.744 g, 2.5 mmol) sequentially incorporated into the growing peptide chain. The resultant pentapeptide hydrazide resin (2.23 g) was then stirred with 44 ml of 50% TFA in CH_2Cl_2 for 30 min and the liberated pentapeptide hydrazide was worked up in a similar manner as described above. The glassy solid obtained was crystallized from DMF by slow addition of methanol: yield 0.38 g (36%); mp 196–198°; $[\alpha]^{25}D$ −0.40° (*c* 1, DMF); NMR spectral data agreed with the expected values.

Anal. Calcd for $C_{62}H_{63}N_7O_9 \cdot H_2O$ (1068.20): C, 69.62; H, 6.13; N, 9.16. Found: C, 69.52; H, 6.00; N, 8.95.

Fmoc-Gly-Ala-Val-Leu-$HNNH_2$ (XIV). Solid-phase synthesis[8–10] with hydrazide resin VIII (1.07 g, 0.74 mmol) using a 2.5-fold excess of Bpoc-Leu-OH (0.683 g), Bpoc-Val-OH (0.657 g), Bpoc-Ala-OH (0.606 g), and Fmoc-Gly-OH (0.55 g) in each of the respective synthetic cycles gave rise to 1.45 g of tetrapeptide hydrazide resin. It was stirred in 30 ml of 50% TFA in CH_2Cl_2 for 30 min and worked up as usual. The gelatinous white solid obtained was crystallized from DMF (15 ml) by slow addition of ethanol (30 ml): yield 0.22 g (49%); mp 220–225° dec; $[\alpha]^{25}D$ −24.25° (*c* 1, DMF); NMR spectral data agreed with the expected value.

Anal. Calcd for $C_{31}H_{42}N_6O_6 \cdot \frac{1}{2}H_2O$ (604.7): C, 61.73; H, 7.29; N, 13.89. Found: C, 61.99; H, 7.23; N, 13.90.

Rate of Cleavage of Aminoacyl Resin Anchoring Bonds by Different Concentrations of Trifluoroacetic Acid. The hydrazide resin VIII (1.0 g, 0.71 mmol) was allowed to react with Bpoc-Ala-OH (0.66 g, 2.0 mmol) and DCC (0.412 g, 20 mmol) in CH_2Cl_2 (10 ml) for 120 min. The ensuing Bpoc-Ala-HNNH-COO-$C(CH_3)_2CH_2CH_2C_6H_4OCH_2C_6H_4$-Resin (1.24 g) contained 0.56 mmol/g aminoacyl hydrazide (Anal. N, 2.32). Part of the sample (0.5 g) was stirred in 5 ml of CH_2Cl_2 for a few minutes and then mixed with 5 ml of 1% trifluoroacetic acid in CH_2Cl_2 or 5 ml of trifluoroacetic acid. At various time intervals, aliquots were withdrawn and the resin in each sample washed immediately with CH_2Cl_2, DMF, and methanol. Each individual resin sample was then examined by ir spectrophotometry and also by nitrogen analysis. The rate of disappearance of the carbonyl band relative to the polystyrene band at 1600 cm^{-1} was taken as the rate of cleavage of the anchoring bond. The rate of decrease in nitrogen content of the resin was another indication for the rate of cleavage of the same anchoring bond. Both the results of ir and nitrogen analysis agreed with each other rather well. The nitrogen analysis data are plotted in Figure 1A. The anchoring bond is quite stable in 0.5% trifluoroacetic acid but rapidly cleaved by 50% trifluoroacetic acid. Similar experiments were conducted with Bpoc-Ala-$OCH_2C_6H_4$-

$OCH_2C_6H_4$-Resin (0.53 mmol/g)[10] with the results shown in Figure 1B. The stabilities of the anchoring bonds in these resins are similar.

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Registry No.—I, 9003-70-7; VIII, 54276-63-0; IX, 54276-64-1; X, 51095-58-0; XI, 54276-65-2; XII, 54276-66-3; XIII, 54276-67-4; XIV, 54276-68-5; potassium acetate, 127-08-2; Boc-Ala-OH, 15761-38-3; Boc-Ala-$OCH_2C_6H_5$, 51814-54-1; Boc-Gly-OH, 4530-20-5; Boc-Gly-$OCH_2C_6H_5$, 54244-69-8; 4-(*p*-hydroxyphenyl)-2-butanone, 5471-51-2; phenyl chloroformate, 1885-14-9; 2-nitrobenzyl-*p*-nitrophenyl carbonate, 54276-69-6; 2-nitrobenzyl alcohol, 612-25-9; *p*-nitrophenyl chloroformate, 7693-46-1; 2-nitrobenzyloxycarbonylglycine, 30007-79-5; glycine, 56-40-6; Boc-Thr(Bzl)-$OCH_2C_6H_5$, 54276-70-9; Boc-Tyr(Bzl)-OH, 2130-96-3; Boc-Phe-OH, 13734-34-4; Boc-Tyr(Cl_2Bzl)-OH, 40298-71-3; Boc-Tyr(Cl_2Bzl)-$OCH_2C_6H_5$, 54244-64-3; Boc-Val-OH, 13734-41-3; Aoc-Arg(Tos)-OH, 54244-59-6; pGlu-Ser(Bzl)-Gly-NH_2, 54276-71-0; pyroglutamic acid, 16891-48-8; Boc-Ser(Bzl)-OH, 23680-31-1; pGlu-Gln-Ala-NH_2, 38357-81-2; Boc-Gln-OH, 13726-85-7; Bpoc-Leu-OH, 18634-99-6; Bpoc-Ala-OH, 23631-89-2; Bpoc-Val-OH, 25692-88-0; Bpoc-Thr(Bzl)-OH, 47733-62-0; Bpoc-Tyr(Bzl)-OH, 25692-91-5; Bpoc-Phe-OH, 40099-50-1; Fmoc-Gly-OH, 29022-11-5.

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(24) Abbreviations used: Aoc, *tert*-amyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; Bpoc, 2-(*p*-biphenylyl)-2-propyloxycarbonyl; Bzl, benzyl; Cl_2Bzl, 2,6-dichlorobenzyl; Fmoc, 9-fluorenylmethyloxycarbonyl; Tos, *p*-toluenesulfonyl; Z, benzyloxycarbonyl; Z(2-NO_2), 2-nitrobenzyloxycarbonyl; DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; DMSO, dimethyl sulfoxide.
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A Nuclear Magnetic Resonance Study of Structure in Some Bi- and Tricyclic *N*-Nitrosoamines[1]

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In acyclic *N*-nitrosoamines, the barrier to rotation about the N–N bond, revealed by nuclear magnetic resonance spectra, gives rise to diastereomeric structures. We have prepared a series of bi- and tricyclic *N*-nitrosoamines with a nitrogen in one of the bridges and adjacent to a bridgehead (compounds **3–7**) and investigated their structures by NMR techniques, including the use of europium shift reagent. *N*-Nitrosoamines **3** and **4** were obtained as single compounds, with the nitroso oxygen anti to the vicinal bridgehead, and **5–7** were obtained as mixtures of nonequilibrating diastereomers. The size of the heterocyclic ring influences the geometry between the bridgehead hydrogen (or methyl) and the NNO group sufficiently to account for these differences.

N-Nitrosoamines are interesting as a class of compounds because they are strongly carcinogenic and because their structures are poorly represented by conventional, uncharged valence-bond formulas. This paper is concerned with a structural study of some bi- and tricyclic compounds in which the *N*-nitrosoamine (NNO) group is a member of a ring bridge and is adjacent to a bridgehead position. The geometries of these compounds are considerably more rigid than the acyclic[2] and monocyclic[3] *N*-nitrosoamines which have been studied earlier, and they illustrate substantial 1,5 nonbonded O–H interactions that strongly influence diastereomer ratios.

The nuclear magnetic resonance (NMR) spectrum of *N*-nitrosodimethylamine reveals that the two methyl groups are magnetically nonequivalent up to about 156°.[4] The substantial energy barrier to rotation about the N–N bond[4,5] gives rise in unsymmetrical *N*-nitrosoamines to diastereomeric structures [e.g., (*E*)-**1** and (*Z*)-**1**] which are