Liquid-Phase Syntheses of Protected Peptides on the New 3-Nitro-4-(bromomethyl)benzoylpoly(ethylene glycol) Support

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A further application of the 3-nitro-4-(bromomethyl)benzoylpoly(ethylene glycol) support to the liquid-phase syntheses of protected peptides possessing free C-terminal carboxyl groups is described. The syntheses were performed using the in situ symmetrical anhydride coupling method and the protected peptides were cleaved from the support by photolysis at 350 nm. The five protected peptides Boc-L-Lys(Z)-L-Leu-L-Glu(OBzl)-L-Ala-OH, Boc-L-Lys(Z)-L-Leu-L-Glu(OBzl)-L-Ala-L-Leu-L-Glu(OBzl)-L-Ala-OH, Boc-L-Lys(Z)-L-Ala-L-Glu(OBzl)-L-Ala-L-Leu-L-Glu(OBzl)-L-Ala-OH, Boc-L-Lys(Z)-L-Leu-L-Glu(OBzl)-L-Ala-L-Ala-L-Glu(OBzl)-L-Ala-OH, and Boc-L-Lys(Z)-L-Ala-L-Glu(OBzl)-L-Ala-L-Ala-L-Glu(OBzl)-L-Ala-OH were prepared to be used in the synthesis of sequential polypeptides as models for the double-stranded coiled-coil structure of tropomyosin.

The liquid-phase method of peptide synthesis using soluble poly(ethylene glycol) as the C-terminal protecting group was introduced by Bayer et al. in 1971.[3] This method offers many advantages over the classical and solid-phase methods of peptide synthesis.[4-6] Its major disadvantage, low yields of final peptide obtained by cleavage under drastic conditions such as saponification of hydrazinolysis,[7-9] was overcome by the introduction of a photosensitive 3-nitro-4-(bromomethyl)benzoylpoly(ethylene glycol) support.[10] This support provides a convenient and high yield photolytic method to cleave protected peptides from the poly(ethylene glycol) support and offers increased acid stability of the peptide–polymer ester bond during peptide synthesis. This paper describes a further application of this photosensitive support to the syntheses of fully protected peptides for use in the preparation of sequential polypeptides as models for the double-stranded coiled-coil structure of tropomyosin.

Tropomyosin is involved in the calcium-regulated system of contraction and relaxation.[11] Understanding the essential features required to form the coiled-coil structure should provide a means of determining the detailed molecular interactions that occur between tropomyosin, actin, and troponins, as well as the conformational changes that take place in tropomyosin during the contraction process.

Analysis of the primary structure of tropomyosin led Hodges et al.[12] to propose that the two-stranded coiled-coil was stabilized by hydrophobic residues situated at positions 2 and 5 of the repeating heptad sequence $(X\text{-}N\text{-}X\text{-}X\text{-}N\text{-}X\text{-}X)_n$, where N is a nonpolar residue. This hypothesis was further supported by the complete sequence of tropomyosin[13] and the preliminary conformation studies on the polyheptapeptide $(Lys\text{-}Leu\text{-}Glu\text{-}Ser\text{-}Leu\text{-}Glu\text{-}Ser)_n$.[14] The peptide sequences described in this paper were chosen to determine the effect that varying the size of the hydrophobic residue in positions 2 and 5 has on the formation and stabilization of the coiled-coil structure.

The peptides were synthesized using N^{α}-*tert*-butyloxycarbonyl amino acids under the same conditions employed for conventional liquid-phase peptide synthesis. As shown in Scheme I, 3-nitro-4-(bromomethyl)benzoic acid (I) was esterified to poly(ethylene glycol), average mol wt 6000–7500, via the dicyclohexylcarbodiimide method in the presence and absence of 1-hydroxybenzotriazol (HOBt). The preparation of 3-nitro-4-(bromomethyl)benzoylpoly(ethylene glycol) (IIIA) in the presence of HOBt resulted in a decreased substitution of 3-nitro-4-(bromomethyl)benzoic acid to the poly(ethylene glycol) as indicated by the bromine substitution of 0.076 mmol of Br/g in the presence of HOBt and 0.141 mmol of Br/g in the absence of HOBt. This may be due to a side reaction between the poly(ethylene glycol) and HOBt in the presence of DCC as reported recently by Hemmasi and Bayer.[15] A higher substitution (0.23 mmol of Br/g) of I to the poly(ethylene glycol) could be obtained using the procedure for compound IIIB with a few milliliters of pyridine added as a catalyst.

N^{α}-*tert*-Butyloxycarbonyl amino acids were attached to the support by heating under gentle reflux with diisopropylethylamine in ethyl acetate for 5 days. In the following steps of the synthesis the N^{α}-*tert*-butyloxycarbonyl amino acids were coupled to the growing chain in a stepwise fashion using the symmetrical anhydride method. The coupling step was monitored for completion using the qualitative ninhydrin method. In most cases, the coupling reactions were complete in a single coupling. The peptide–polymer ester bond was stable to the conditions used for peptide synthesis since the peptide substitution remained unchanged. The protected peptides were cleaved from the support by photolysis at a wavelength of 350 nm in anhydrous methanol or DMF in the absence of oxygen and purified by gel filtration on Sephadex LH-20 in methanol–dichloromethane (30:70 v/v).

To achieve maximum cleavage yield, the peptide–PEG was irradiated for 18 h, although 10 h of irradiation was found to be sufficient as shown in the case of the model tetrapeptide Boc-Leu-Ala-Gly-Val-nitro-PEG (Figure 1). The yields obtained for the five peptides prepared in this paper were 95% for Boc-Lys(Z)-Leu-Glu(OBzl)-Ala (IX), 87% for Boc-Lys(Z)-Leu-Glu(OBzl)-Ala-Leu-Glu(OBzl)-Ala (XIV), 96% for Boc-Lys(Z)-Ala-Glu(OBzl)-Ala-Leu-Glu(OBzl)-Ala (XVII), 90% for Boc-Lys(Z)-Leu-Glu(OBzl)-Ala-Ala-Glu(OBzl)-Ala (XXIV), and 92% for Boc-Lys(Z)-Ala-Glu(OBzl)-Ala-Ala-Glu(OBzl)-Ala (XXVII) based on the quantity of alanine on the Boc-Ala-nitro-PEG support as determined by amino acid analysis. The yields of cleaved peptide were determined after removal of the poly(ethylene glycol) by precipitation followed by evaporation of the filtrate to dryness and a water wash of the protected peptide. At this stage, the peptides showed excellent amino acid ratios. Four of the five protected peptides were obtained in an average yield of 92% after photolysis at a concentration of 5 mL of methanol per gram of peptide support. The heptapeptide support XXVI (Scheme II) showed a limited solubility in methanol and was chosen to study the effect of solvent and concentration on the photolysis cleavage yield (Table I). The yield of this peptide was improved by reducing the concentration to 150 mL of methanol per gram peptide support; however, to obtain a 92% yield DMF had to be used as the solvent for photolysis. The protected peptides were purified on a column of Sephadex LH-20 in methanol–dichloromethane. A representative elution profile is shown in Figure 2. Peak 2 gave the analytically pure product and peak 1 contained the

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Scheme I

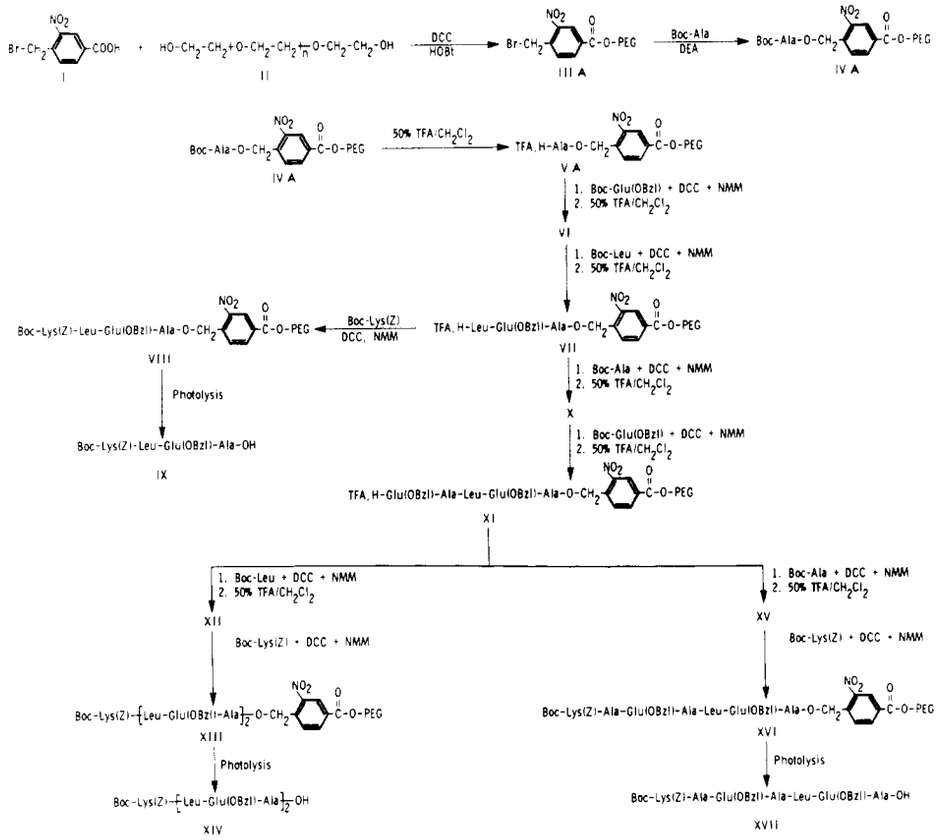


Table I. Effect of Concentration of Peptide–PEG and Solvent on Photolysis Cleavage Yields[a]

solvent volume per gram of peptide–PEG, mL	cleavage yield, %	
	DMF	methanol
7.5	39	21
30	47	35
150	92	47

[a] Determined using peptide–PEG (XXVI).

poly(ethylene glycol) that was not removed during the workup. The LH-20 chromatography gave an average yield of 70%.

To further demonstrate the usefulness of the nitro-PEG support, the tripeptide Boc-Ala-Gly-Val-Nitro-PEG[10] was cleaved from the support with 10% hydrazine hydrate in DMF (v/v) for 15 min. The yield of cleaved peptide hydrazide was 85% based on the quantity of valine on the Boc-Val-nitro-PEG support as determined from amino acid analysis. Based on these results and those obtained from previous work, this new support provides a convenient method for the synthesis of fully protected peptide fragments to be used in the preparation of larger peptides by fragment coupling or polymerization.

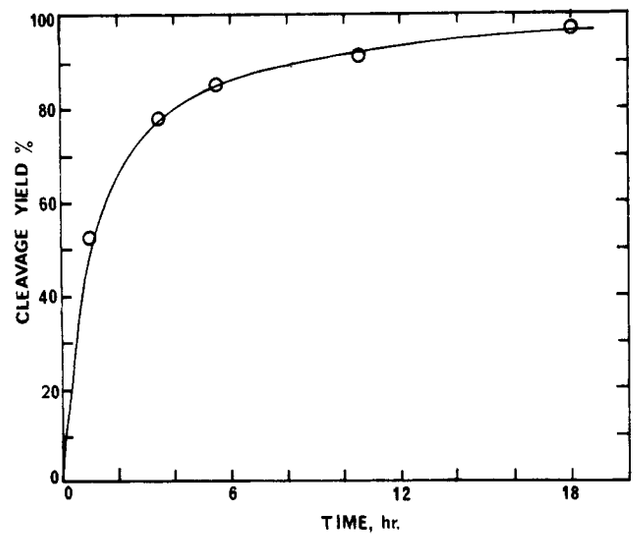
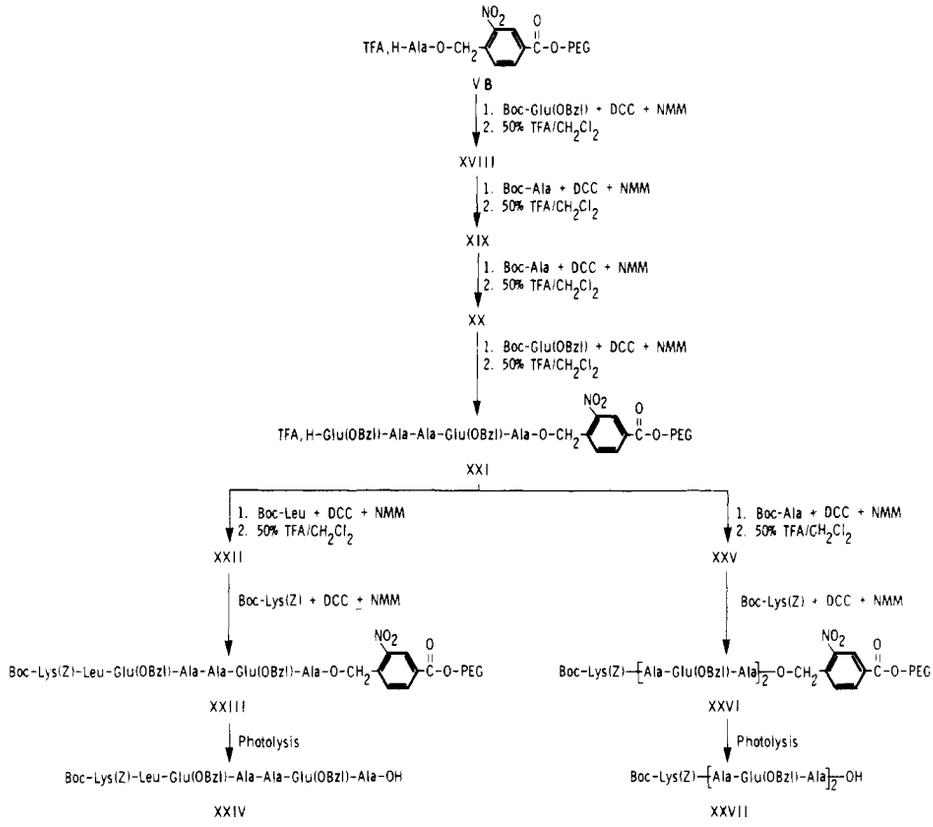


Figure 1. Cleavage yield in percent of Boc-Leu-Ala-Gly-Val obtained by photolysis of the peptide–support as a function of time. The quantity of the cleaved peptide was determined by amino acid analysis. Samples of protected peptide–support (50 mg) were dissolved in 2 mL of absolute methanol.

Experimental Section

Melting points were determined on an Electrochemical melting point apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were recorded with either a Varian Model T-60 or a 270 MHz Brüker spectrometer. The NMR data include the frequency, integration, and assignment of the principal functional groups or characteristic side chains of the five protected peptides (compounds IX, XIV, XVII, XXIV, and XXVII) shown in Schemes I and II. The data refer mainly to the resonances of Boc and Bzl and to the protons of the leucyl, glutamyl, alanyl, and lysyl side chains (β, γ, and δ) which gave the best resolution. Deuteriochloroform or deuterated CH_3OH (99%) was used as solvent with tetramethylsilane as an internal reference. Elemental analyses were performed at the Microanalytical Laboratory, Department of Chemistry, University of Alberta. IR spectra were recorded with a Beckman IR 12 spectrometer using KBr pellets with 0.5% of sample. All photolyses were done in a RPR 208 preparative reactor (Rayonet, The Southern New England Ultraviolet Co., Middletown, Conn.) equipped with RPR 3500-Å lamps. With the reactor at room temperature, the air temperature surrounding the sample was maintained at 32 °C by an electric fan. Amino acid analyses were obtained on a Durrum Model D-500 high-pressure automatic analyzer after hydrolysis of the samples with 6 N HCl in sealed evacuated tubes at 110 °C for 24 h for cleaved peptides and 36 h for peptide–PEG support. TLC was performed with precoated silica gel plates with a fluorescent indicator obtained from Eastman Kodak

Scheme II



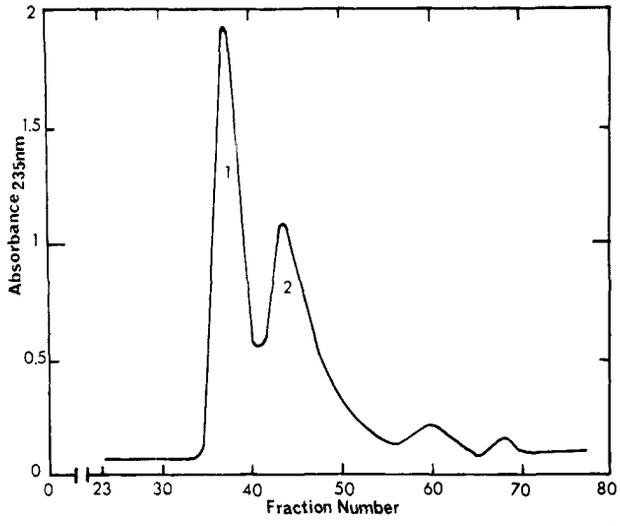


Figure 2. Chromatographic purification of synthetic heptapeptide XVII, Boc-Lys(Z)-Ala-Glu(OBzl)-Ala-Leu-Glu(OBzl)-Ala-OH, on Sephadex LH-20. The column, 2.2 × 40 cm, was equilibrated with methanol–dichloromethane (3:7 v/v), and a sample of 24 mg dissolved in 0.5 mL was applied. The flow rate was 12 mL/h, and 2-mL fractions were collected. The effluent was monitored by the absorbance of 235 nm.

(No. 6060) or Analtech silica gel GF plates (0.25 mm). The following solvent systems were used: 1-butanol–acetic acid–water, 3:1:1 (system A); 1-butanol–concentrated NH_4OH, 7:3 (system B); and 1-butanol–acetic acid–water, 4:1:1 (system C). The compounds were visualized directly under ultraviolet light or by spraying with ninhydrin in acetone followed by heating after removal of the Boc group by exposure to HCl fumes. The peptides were homogeneous in the solvent systems described when 300 μg of peptide was applied to the thin-layer plate.

The following abbreviations were used: DCC, *N,N'*-dicyclohexylcarbodiimide; DCU, dicyclohexylurea; DEA, diisopropylethylamine; NMM, *N*-methylmorpholine; HOBt, 1-hydroxybenzotriazole; Boc, N^{α}-*tert*-butyloxycarbonyl; PEG, poly(ethylene glycol); nitro-PEG, 3-nitro-4-(bromomethyl)benzoylpoly(ethylene glycol); THF, tetrahydrofuran; DMF, *N,N*-dimethylformamide.

The general procedures of deprotection, coupling, photolysis, and peptide purification are described below along with the characterization of the five finished protected peptides.

Deprotection Procedure. All amino acids were protected on the α-amino position with the Boc group. The Boc groups were removed at each cycle of the synthesis by treatment for 30 min with 50% TFA–CH_2Cl_2 (v/v) using 10 mL of this solution per gram of peptide support. The volume of the solution was then reduced by flash evaporation to an oil which was triturated with anhydrous ether to give a precipitate. After standing for 1 h at −20 °C, the precipitate was filtered, washed thoroughly with ether, and dried in vacuo.

Coupling Procedure. All couplings were made via the in situ symmetrical anhydride method, using a 5-fold molar excess of Boc amino acid and a 2.5-fold molar excess of DCC. The Boc amino acid was dissolved in 15 mL of dichloromethane, and the solution was cooled to 0 °C. A solution of DCC in 15 mL of dichloromethane was prepared and cooled to 0 °C. Both solutions were combined and allowed to stand at 0 °C for 1 h. This solution containing the symmetrical anhydride of the Boc amino acid was filtered directly into a flask containing the deprotected peptide support dissolved in 7–10 mL of dichloromethane per gram of peptide support. NMM was added to neutralize the trifluoracetate salt (pH 7.5–8.0 as measured on moistened indicator paper). After stirring the reaction mixture for 1 h at room temperature, the pH was readjusted to 7.5–8.0 if necessary by further addition of NMM and the reaction mixture was stirred for an additional 1–3 h. The solution was reduced to a small volume by evaporation in vacuo. The peptide–PEG support was precipitated by the slow addition of anhydrous ether, filtered, washed with ether, and dried in vacuo. A small sample was taken for TLC in system A to check for the complete removal of uncoupled Boc amino acid during precipitation.

The extent of coupling was monitored by a qualitative ninhydrin test. A sample of the peptide support (10–20 μmol of peptide) in 1 mL of water was added to 1 mL of ninhydrin reagent (1 L of 4 N NaAc buffer (pH 5.5), 1 L of methylcellosolve, 80 g of ninhydrin, and 7.6 mL of a 20% solution of titanium trichloride and the mixture was heated at 90 °C for 20 min. The coupling was judged to be complete when no blue color could be detected on visual observation. A second coupling was carried out if this test was positive.

Photolytic Cleavage of the Peptide from the Support. The

peptide support was dissolved in absolute methanol (5 mL/g of support). The solution was deaerated with nitrogen for 2 h and irradiated for 18 h at 350 nm. After irradiation, the solution containing the cleaved peptide, the peptide–PEG, and the PEG was diluted with absolute methanol to a volume of 1 L. It was allowed to stand at −20 °C for 3 h and then rapidly filtered. This procedure was repeated twice, and the combined filtrates containing the protected peptide were evaporated to dryness. The crude product was twice suspended in water (5 mL/g of peptide support used for photolysis) and centrifuged, and the supernatant was removed by decantation and discarded. The precipitate containing the protected peptide was further purified by Sephadex LH-20 chromatography.

Cleavage of the Peptide from the Support by Hydrazinolysis. The peptide support, Boc-Ala-Gly-Val-PEG[10] (59 mg containing 0.113 mmol of peptide per gram), was dissolved in 25 mL of DMF, and 3 mL of hydrazine hydrate was added with constant stirring. The solution was stirred for 15 min at room temperature and evaporated to dryness. The residue was dissolved in 20 mL of ethanol, evaporated to dryness, and redissolved in 10 mL of ethanol, and 90 mL of anhydrous ether was added. The mixture was left at −20 °C for 2 h and filtered at 4 °C. The filtrate containing the cleaved peptide hydrazide was evaporated to dryness. The cleavage yield was 85%, as determined by quantitative amino acid analysis.

Chromatographic Purification on Sephadex LH-20. The protected peptide was chromatographed on Sephadex LH-20 in a 2.2 × 40 cm column equilibrated with methanol–dichloromethane (3:7 v/v). The flow rate was 12–15 mL/h, and fractions of 2 mL were collected. The complete removal of PEG and peptide–PEG from the protected peptide was determined on TLC (system A) and by IR spectroscopy from the disappearance of the intensive and characteristic ether band of PEG at 1100 cm^{-1}.

3-Nitro-4-(bromomethyl)benzoic Acid (I). This compound was prepared by adding 4-(bromomethyl)benzoic acid (60 g, 0.23 mol) to 500 mL of 90% HNO_3 (white fuming) as previously described[16] to yield 64 g (89%), mp 132–135 °C (lit.[15] mp 125–126 °C).

Anal. Calcd for $C_8H_6NBrO_4$: C, 36.95; H, 2.32; N, 5.38; Br, 30.73. Found: C, 36.67; H, 2.20; N, 5.21; Br, 30.55.

3-Nitro-4-(bromomethyl)benzoylpoly(ethylene glycol) (IIIA). 3-Nitro-4-(bromomethyl)benzoic acid (13 g, 50 mmol) and HOBt (7.65 g, 50 mmol) were dissolved in 100 mL of THF and added to poly(ethylene glycol) 6000 (30 g; containing a total of 10 mmol of hydroxyl groups). DCC (10.5 g 50 mmol) in 200 mL of dichloromethane was added, and the reaction mixture was stirred at room temperature for 24 h. The mixture was filtered to remove precipitated DCU. The above quantities of 3-nitro-4-(bromomethyl)benzoic acid, DCC, and HOBt were added to the filtrate, and the mixture was stirred for an additional 3 days. The precipitate of DCU was filtered off, the filtrate was concentrated in vacuo to a small volume, and the product was precipitated by the addition of ether. The product was filtered, washed thoroughly with ether, recrystallized from methanol by the slow addition of ether with rapid stirring, filtered, washed with ether, and dried under vacuum to yield 29.5 g: Br, 1.22 % (0.076 mmol of Br/g).

3-Nitro-4-(bromomethyl)benzoylpoly(ethylene glycol) (IIIB). 3-Nitro-4-(bromomethyl)benzoic acid (13 g, 50 mmol) was added to poly(ethylene glycol) 6000 (30 g; containing a total of 10 mmol of hydroxyl groups) followed by DCC (10.5 g, 50 mmol) in 250 mL of dichloromethane. The reaction conditions and isolation of compound IIIB were the same as those previously described for compound IIIA: yield 29 g; Br, 2.25 % (0.141 mmol of Br/g).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-L-alanyl-*O*-nitrobenzoylpoly(ethylene glycol) (IVA).** 3-Nitro-4-(bromomethyl)benzoylpoly(ethylene glycol) IIIA (17 g) was dissolved in 70 mL of ethyl acetate, followed by 3.024 g (16 mmol) of *N*$^{\alpha}$-*tert*-butyloxycarbonyl-L-alanine and 2.08 g (16 mmol) of DEA. The reaction mixture was heated gently at reflux for 5 days. After cooling the solution to room temperature, 400 mL of ether was added and the precipitate was filtered and washed with ether until no traces of *N*$^{\alpha}$-*tert*-butyloxycarbonyl-L-alanine were detected by TLC on silica gel (solvent system A). R_f values for compound IVA and *N*$^{\alpha}$-Boc-L-alanine were 0 and 0.56, respectively. The precipitate was dried in vacuo to give 17.05 g of compound IVA. The substitution of alanine on the support was 0.044 mmol/g of compound IVA, as determined by quantitative amino acid analysis after a 36-h hydrolysis in 6 N HCl at 110 °C. Compound IVA was deprotected as previously described to yield 17.0 g of compound VA.

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-L-alanyl-*O*-nitrobenzoylpoly(ethylene glycol) (IVB).** The esterification of Boc-Ala (2.84 g, 15 mmol) to 3-nitro-4-(bromomethyl)benzoylpoly(ethylene glycol) IIIB (15 g) was carried out in the presence of 1.94 g (15 mmol) of DEA using the same procedure as described for compound IVA. The substitution of alanine on the support was 0.092 mmol/g of compound IVB, as determined by quantitative amino acid analysis. Compound IVB was deprotected as previously described to yield 14.7 g of compound VB.

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-*N*$^{\epsilon}$-carbobenzoxy-L-lysyl-L-leucyl-γ-benzyl-L-glutamyl-L-alanine (IX).** Compound VIII (4.0 g, 0.176 mmol) was dissolved in 20 mL of absolute methanol and cleaved from the support as previously described to yield 130 mg (95%) as determined by quantitative amino acid analysis. Further purification on Sephadex LH-20 gave compound IX: yield 100 mg (85%); mp 140–143 °C; R_f (system C) 0.86; NMR analysis in $CDCl_3$–Me_4Si (1%) showed δ 0.95 (6 H, multiplet, leucine methyls), 1.35 (3 H, doublet, alanine methyl), 1.43 (9 H, singlet, Boc methyls), 5.10 (4 H, singlet, methylenes of Bzl and Z), and 7.34 (10 H, singlet, aromatic). Amino acid analysis: Glu, 0.96; Ala, 1.02; Leu, 0.96; Lys, 1.05.

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-*N*$^{\epsilon}$-carbobenzoxy-L-lysyl-L-leucyl-γ-benzyl-L-glutamyl-L-alanyl-L-leucyl-γ-benzyl-L-glutamyl-L-alanine (XIV).** Compound XIII (5.9 g, 0.26 mmol of peptide) was dissolved in 25 mL of absolute methanol and irradiated at a wavelength of 350 nm for 18 h as previously described. The yield obtained after photolysis was 265 mg (87%). The peptide was purified by Sephadex LH-20 chromatography to yield 186 mg (70%) of compound XIV: mp 218 °C; R_f (system A) 0.76, R_f (system B) 0.41; NMR analysis in CD_3OD showed δ 0.92 (12 H, 2 doublets, leucine methyls), 1.38 (6 H, doublet, alanine methyls), 1.46 (9 H, singlet, Boc methyls), 5.10 (6 H, singlet, methylenes of Bzl and Z), and 7.34 (15 H, singlet, aromatic). Amino acid analysis: Glu, 2.07; Ala, 1.91; Leu, 2.02; Lys 0.99.

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-*N*$^{\epsilon}$-carbobenzoxy-L-lysyl-L-alanyl-γ-benzyl-L-glutamyl-L-alanyl-L-leucyl-γ-benzyl-L-glutamyl-L-alanine (XVII).** Compound XVI (5.1 g, 0.224 mmol of peptide) was dissolved in 25 mL of absolute methanol and irradiated for 18 h, and compound XVII was isolated as previously described to yield 246 mg (96%). This product was purified by Sephadex LH-20 chromatography to yield 186 mg (76%) of pure compound XVII: mp 227 °C dec; R_f (system A) 0.79, R_f (system B) 0.42; NMR analysis in CD_3OD showed δ 0.94 (6 H, 2 doublets, leucine methyls), 1.40 (9 H, doublet, alanine methyls), 1.46 (9 H, singlet, Boc methyls), 5.08 (6 H, singlet, methylenes of Bzl and Z), and 7.34 (15 H, singlet, aromatic). Amino acid analysis: Glu, 1.94; Ala, 2.99; Leu, 1.02; Lys, 1.05.

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-*N*$^{\epsilon}$-carbobenzoxy-L-lysyl-L-leucyl-γ-benzyl-L-glutamyl-L-alanyl-L-alanyl-γ-benzyl-L-glutamyl-L-alanine (XXIV).** Compound XXIII (5.2 g, 0.48 mmol of peptide) was dissolved in 25 mL of absolute methanol and irradiated for 18 h, and compound XXIV was isolated as previously described to yield 482 mg (90%). This product was purified on Sephadex LH-20 to yield 361 mg (74%) of pure compound XXIV: mp 227–230 °C dec; R_f (system A) 0.71, R_f (system B) 0.29; NMR analysis in CD_3OD showed δ 0.95 (6 H, multiplet, leucine methyls, 1.32 (9 H, doublet, alanine methyls), 1.42 (9 H, singlet, Boc methyls), 4.81 (6 H, singlet, methylenes of Bzl and Z), and 7.3 (15 H, singlet, aromatic). Amino acid analysis: Glu, 2.01; Ala, 2.92; Leu, 1.01; Lys, 1.05.

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-*N*$^{\epsilon}$-carbobenzoxy-L-lysyl-L-alanyl-γ-benzyl-L-glutamyl-L-alanyl-L-alanyl-γ-benzyl-L-glutamyl-L-alanine (XXVII).** Compound XXVI (6.7 g, 0.62 mmol of peptide) was dissolved in 25 mL of absolute methanol and irradiated for 18 h, and compound XXVII was isolated as previously described to yield 130 mg (19%). This product was purified on Sephadex LH-20 to yield 84 mg (65%) of pure compound XXVII: mp 234 °C dec; R_f (system A) 0.78, R_f (system B) 0.36; NMR analysis in CD_3OD showed δ 1.38 (12 H, doublet, alanine methyls), 1.44 (9 H, singlet, Boc methyls), 5.10 (6 H, singlet, methylenes of Bzl and Z), and 7.42 (15 H, singlet, aromatic). Amino acid analysis: Glu, 2.05; Ala, 3.93; Lys, 1.02.

Registry No.—I, 555715-03-2; II, 25322-68-3; III, 67316-51-2; IV, 67271-86-7; V, 67315-52-0; VII, 67271-87-8; IX, 67271-88-9; XIII, 67271-85-6; XIV, 67316-54-5; XVI, 67271-84-5; XVII, 67271-89-0; XXIII, 67316-52-3; XXIV, 67271-90-3; XXVI, 67271-83-4; XXVII, 67271-91-4; BOC-Ala-Gly-Val-PEG, 67271-82-3; 4(bromomethyl)-benzoic acid, 6232-88-8; *N*$^{\alpha}$-*tert*-butyloxycarbonyl-L-alanine, 15761-38-3; BOC-Leu-Ala-Gly-Val-OH, 61165-83-1.

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Rapid Removal of Protecting Groups from Peptides by Catalytic Transfer Hydrogenation with 1,4-Cyclohexadiene

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1,4-Cyclohexadiene is a very effective hydrogen donor for catalytic transfer hydrogenation. *N*-Benzyloxycarbonyl, benzyl ester, and benzyl ether (tyrosine) protecting groups can be rapidly removed at 25 °C with 1,4-cyclohexadiene and 10% palladium–carbon catalyst. Removal of the N^{im}-benzyl group from histidine, the N^{g}-nitro group from arginine, and the benzyl ether groups from serine and threonine can be carried out at 25 °C using palladium black as catalyst. Cleavage of *N*-benzyloxycarbonyl groups from sulfur-containing amino acids was also achieved by catalytic transfer hydrogenation with 1,4-cyclohexadiene. *tert*-Butyl-derived protecting groups were completely stable under these conditions. The scope of the 1,4-cyclohexadiene-catalyzed transfer hydrogenation for the removal of benzyl-derived protecting groups used in peptide synthesis was examined.

Recent publications[1,2] from two laboratories described the use of catalytic transfer hydrogenation for the removal of several benzyl-type protecting groups used in peptide synthesis. Good yields of homogeneous and nonracemized products were obtained when cyclohexene was used as hydrogen donor at temperatures of >65 °C (refluxing methanol or ethanol). However, in certain cases, especially when *tert*-butyl-derived protecting groups are also present, the danger of thermal decomposition at elevated temperatures might discourage the use of catalytic transfer hydrogenation.

We have examined other hydrogen donors and now report that 1,4-cyclohexadiene is a much more effective donor and can be used to carry out catalytic transfer hydrogenations at 25 °C in the presence of 10% palladium–charcoal. Under these conditions, removal of *N*-benzyloxycarbonyl, benzyl ester, and tyrosine benzyl ether protecting groups was complete within 2 h and good yields of analytically pure amino acids and peptides were obtained directly. The more efficient palladium-black catalyst was required for cleavage of the N^{im}-benzyl group from histidine, N^{g}-nitro group from arginine, and benzyl ether groups from serine and theonine at 25 °C. The scope of the catalytic transfer hydrogenation reaction was evaluated with respect to hydrogen donor, solvent, concentration, catalyst, and reaction temperature.

Results and Discussion

Nature of the Donor. Catalytic transfer hydrogenation has been used for the reduction of a variety of functional groups (including olefins, acetylenes, imines, hydrazones, azo, and nitro compounds).[3] The availability and reactivity of cyclohexene have rendered this reagent a preferred hydrogen donor.[4,5] The rapid disproportionation reported[6] for 1,3-cyclohexadiene and 1,4-cyclohexadiene prompted us to examine their effectiveness as hydrogen donors for the catalytic transfer hydrogenolysis of benzyl-derived protecting groups used in peptide synthesis. We observed that transfer hydrogenation of *N*-benzyloxycarbonyl-L-alanine in ethanol at 25 °C in the presence of 10% palladium–carbon and 1,4-cyclohexadiene required only 1.5 h for complete deprotection. Under the same conditions, using 1,3-cyclohexadiene as the hydrogen donor, the required reaction time for complete removal of the benzyloxycarbonyl group was 8 h. When cyclohexene was used, there was no deprotection, even after 24 h at 25 °C.

Studies were also carried out to determine the excess of hydrogen donor required for optimum deprotection. An excess of 5–10 equiv of 1,4-cyclohexadiene (per protecting group) is ideal. The rate of transfer hydrogenolysis decreased substantially when only 1 equiv of hydrogen donor was used. On the other hand, a large excess of 1,4-cyclohexadiene (50 equiv) produced only a marginal increase in the rate of reaction compared to that observed when 5–10 equiv were used.

Solvent and Concentration. Most of the solvents employed for catalytic hydrogenolysis of peptides are also useful for the catalytic transfer hydrogenation procedure. Glacial acetic acid was the most effective solvent. Transfer hydrogenation of *N*-benzyloxycarbonyl-L-alanine at 25 °C in the presence of 10% palladium–carbon and 10 equiv of 1,4-cyclohexadiene required only 45 min for complete deprotection in glacial acetic acid. Other solvents were also useful for transfer hydrogenation but required somewhat longer reaction times for complete deprotection: ethanol (1.5 h), dimethylacetamide (3 h), methanol (3.5 h), and dimethylformamide (5 h). The following solvents were impractical for catalytic transfer hydrogenation since only partial deprotection was observed at 25 °C even after prolonged periods of reaction: hexamethylphosphoramide, trifluoroethanol, phenol, trifluoroacetic acid, tetrahydrofuran, dimethyl sulfoxide, and isopropyl alcohol.

Literature[1,2] reports on inhibition of catalytic transfer hydrogenation by sulfur-containing amino acids are conflicting. We have observed that transfer hydrogenation in ethanol (using palladium-black catalyst) removed the *N*-benzyloxycarbonyl group from methionine, but not from *S*-benzylcys-

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