TABLE III

$$\text{Ia–d} + \text{III} \xrightarrow[\text{solvent}]{\text{NaH}} \text{VI} + (C_6H_5)_3PO$$

Compd (mol)	NaH, mol	Temp, °C	Solvent	Time	Product (yield, %)	Bp, (mm) or mp, °C	Refractive index	—Calcd, %— C	H	—Found, %— C	H
Ia (0.0721)	0.0625	Reflux	Acetonitrile	5 days	VIa (57.5)	91–92 (0.85)[a]	$n^{29}D$ 1.5860				
IIb (0.016)		155–160 (2.0 mm)	Fusion		VIb (27.2)	125–126		61.01	3.99	60.78	4.03
Ic (0.064)	0.064	100	DMF	3 days	VIc (29)[b]	79.5–80 (1.0)		64.88	4.23	64.81	4.07
IId (0.06)		Reflux	Acetonitrile	24 hr	VId (71)[c]	63–67 (3.0)[d]	$n^{20}D$ 1.5886				
VIII (0.065)	0.054	Reflux	Acetonitrile	5 days	IX (13.7)	34–35[e]					

[a] Lit.[8] bp 115–118° (1.0 mm) bath temp, $n^{16}D$ 1.5917. [b] In an unsuccessful attempt to obtain VIc, the only product was 1.1 g (9%) of a dimeric acetal, mp 176–178°. Analysis and ir and nmr spectra are in agreement with assigned structure for anhydro di(chlorosalicylaldehyde) (lit.[16] mp 172°). [c] The acetonitrile is distilled off. Remains are distilled to yield product. [d] Lit.[11] bp 49.5–50.0° (1.0 mm), $n^{20}D$ 1.5879. [e] Purification by sublimation at 60° (0.05 mm) (lit.[9] mp 40–41.5°).

heated to reflux for 5 days, cooled to room temperature, poured into 1 l. of a 10% sodium hydroxide solution, and extracted with ether. The ethereal extract was dried ($MgSO_4$), concentrated, and distilled affording 5.75 g (57.5%) of 8-methoxy-2H-1-benzopyran (VIa): bp 91–92° (0.85 mm); $n^{29}D$ 1.5860 (lit.[8] bp 115–118° (1.0 mm), bath temperature; $n^{16}D$ 1.5917). The ir and nmr data may be found in Table II; the reaction conditions employed and, in case the product is a new compound, the analyses may be found in Table III.

Fused Reaction. Preparation of 6-Nitro-2H-1-benzopyran (VIb).—5-Nitrosalicylaldehyde (5 g, 0.03 mol) was added slowly to a stirred mixture of 2.9 g of a sodium hydride dispersion in mineral oil (52% NaH, 1.5 g, 0.0625 ml) and 100 ml of ether. After the gas evolution abated, the reaction mixture was cooled to ice-bath temperature and filtered under a nitrogen cover. The precipitated sodium salt of nitrosalicylaldehyde was washed with cold ether and dried over night. Salt III (6.3 g, 0.017 mol) was intimately blended with 3.0 g (0.016 mol) of the sodium salt of IIb and heated *in vacuo* in a sublimer. At 155–160° (2.0 mm), a yellow solid was collected on the cold finger of the sublimer which upon recrystallization from methanol afforded 0.76 g (27.2%) of 6-nitro-2H-1-benzopyran, mp 125–126°. Analysis is found in Table III.

(16) W. P. Bradley and F. B. Dains, *Amer. Chem. J.*, **14**, 293 (1892).

The preparation of 4-methyl-2H-1-benzopyran and 4-phenyl-2H-1-benzopyran has been attempted by two workers more than ten times (in each case) using both the synthetic procedures described above.

In the case of the 4-methyl-2H-1-benzopyran, the vapor phase chromatogram showed a small peak of a compound boiling higher than DMF but lower than the starting material. The amount of material was too small to allow identification of this product. A trace amount of triphenylphosphine oxide was identified by melting point and ir spectrum. The presence of it indicates that the products are formed but only in very small yields.

In the case of 4-phenyl-2H-1-benzopyran, the vapor phase chromatogram also showed a peak which would be assumed to originate from the product, but the amount was too small to allow positive identification. A trace amount of isolated triphenylphosphine oxide hints toward a reaction in very low yield.

Registry No.—VIa, 16336-25-7; VIb, 16336-26-8; VIc, 16336-27-9; VId, 254-04-6; IX, 229-80-1.

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The Selective Reduction of the Carbobenzoxy Group in Carbobenzoxyamino Acid and Peptide *p*-Nitrophenyl Esters

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The N-carbobenzoxy group of *p*-nitrophenyl esters of N-carbobenzoxyamino acids and N-carbobenzoxy peptides can be removed by catalytic hydrogenation in the presence of 1 equiv of hydrochloric acid without noticeable reduction of the nitro group. This method can be used for preparing oligo peptides by Goodman's "backing-off" procedure and for preparing polyamino acids and sequential polypeptides when the *t*-butyl ester groups are present.

Removal of the N-carbobenzoxy group from N-carbobenzoxyamino acid and N-carbobenzoxy peptide *p*-nitrophenyl esters is usually achieved by treatment with hydrogen bromide in glacial acetic acid,[1] since catalytic hydrogenation is expected to reduce the nitro group under the usual conditions. In the case of trifunctional amino acids, where, in addition to the N-carbobenzoxy group, an acid-sensitive group such as the *t*-butyl group[2] is also present, the hydrogen bromide method cannot be used.

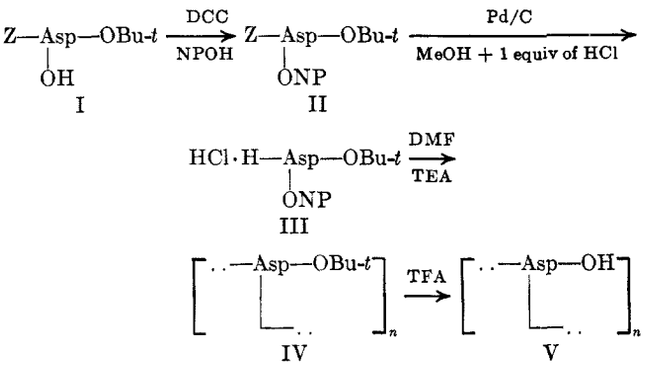
In this paper, a method is described for the selective removal of the N-carbobenzoxy group by catalytic hydrogenation from N-carbobenzoxyamino acid and N-carbobenzoxy peptide *p*-nitrophenyl esters, without noticeable reduction of the nitro group. This method can be used with the "backing-off" procedure of Goodman[3] as well as for preparing C-activated peptides or amino acids where *t*-butyl containing trifunctional amino acids are present. These C-activated peptides and amino acids can, in turn, be polymerized to sequential polypeptides and polyamino acids.

This selective catalytic hydrogenation procedure was studied on N-carbobenzoxyglycine *p*-nitrophenyl ester, N-carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester, N-carbobenzoxy-α-*t*-butyl-L-glutamic acid *p*-nitrophenyl ester, and N-carbobenzoxy-L-phenylalanylglycine *p*-nitrophenyl ester. The procedure was best illustrated by the preparation of α-*t*-butyl-L-aspartic acid *p*-nitrophenyl ester hydrochloride and the polym-

(1) D. Ben-Ishai and A. Berger, *J. Org. Chem.*, **17**, 1564 (1952).

(2) G. W. Anderson and F. M. Callahan, *J. Amer. Chem. Soc.*, **82**, 3359 (1960).

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



erization of this active ester to the corresponding poly-amino acid derivative.

N-carbobenzoxy-L-aspartic acid *p*-nitrophenyl ester (II), prepared from the corresponding free acid I by the carbodiimide[4,5] method, was hydrogenated with pre-hydrogenated palladium catalyst in a methanolic suspension in the presence of 1 equiv of hydrogen chloride. The rate of removal of the N-carbobenzoxy group under these conditions is much faster than the rate of reduction of the nitro group. The reaction was usually complete within 5 min.[6] It was important to complete the reaction and to isolate the active ester hydrochloride within the shortest period of time to avoid side reactions. The hydrochloride salt III, which was obtained in 90% yield, analyzed correctly and showed strong peaks which are characteristic for *p*-nitrophenyl ester, *t*-butyl ester, and nitro groups in its infrared spectrum. Further evidence for structure III was provided by polymerization of this compound to poly-α-*t*-butyl-L-aspartate (IV). Polypeptide IV, which has a molecular weight of 17,000 as determined by the sedimentation equilibrium[7] method was converted into poly-β-L-aspartic acid (V) by the usual treatment with trifluoroacetic acid. After extensive dialysis and lyophilization, the poly-β-L-aspartic acid was obtained as a white fluffy material which had an infrared spectrum identical with a sample prepared by another method.[8,9] The molecular weight was 8000 as determined by the sedimentation equilibrium[7] method.

The selective removal of the N-carbobenzoxy group from N-carbobenzoxyglycine *p*-nitrophenyl ester,[10] N-carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester,[11] and N-carbobenzoxy-α-*t*-butyl-L-glutamic acid *p*-nitrophenyl ester was carried out as described above. Under normal catalytic hydrogenation conditions, when much more than half of the theoretical amount of hydrogen was absorbed (see Experimental Section), N-carbobenzoxyglycine *p*-nitrophenyl ester gave glycine *p*-aminophenyl ester dihydrochloride.

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

(5) H. C. Khoranna, *Chem. Ind.* (London) 1087 (1955).

(6) The reaction was complete when it was observed that one-half of the theoretical amount of hydrogen had been absorbed. During this time the same volume of carbon dioxide was liberated; however, it is partially soluble in the solvent system; therefore the measured amount of hydrogen absorbed is not a true indication of the completeness of the reaction.

(7) H. K. Schachman, "Ultracentrifuge in Biochemistry," Academic Press Inc., New York, N. Y., 1955.

(8) J. Kovacs, R. Ballina, and R. L. Rodin, *Chem. Ind.* (London), 1955 (1963).

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(10) M. Bodanszky, *Nature*, **175**, 685 (1955); B. Iselin, W. Rittel, P. Sieber, and R. Schwyzer, *Helv. Chim. Acta*, **40**, 373 (1957).

(11) M. Bodanszky and V. du Vigneaud, *J. Amer. Chem. Soc.*, **81**, 6072 (1959).

The dipeptide, N-carbobenzoxy-L-phenylalanylglycine *p*-nitrophenyl ester, prepared from N-carbobenzoxy-L-phenylalanine and glycine *p*-nitrophenyl ester hydrochloride or glycine *p*-nitrophenyl ester hydrobromide using the mixed anhydride coupling procedure, gave, after the above-described catalytic hydrogenation, the dipeptide active ester hydrochloride in 80% yield.

Experimental Section

All melting points are uncorrected. The microanalyses were carried out by Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Infrared spectra were determined in potassium bromide pellets using a Perkin-Elmer Model 137 spectrophotometer.

N-Carbobenzoxy-α-*t*-butyl-L-aspartic Acid *p*-Nitrophenyl Ester (II).—A suspension of 45 g (0.14 mol) of N-carbobenzoxy-α-*t*-butyl-L-aspartic acid,[12] 31.5 g (0.153 mol) of dicyclohexylcarbodiimide, and 19.4 g (0.139 mol) of *p*-nitrophenol in 250 ml of methylene chloride was stirred at 0° for 3 hr and overnight at room temperature. Ten drops of acetic acid were added and the mixture was stirred for an additional 30 min. The dicyclohexylurea was filtered and the filtrate was concentrated under reduced pressure. The product was an oil (61 g) which solidified after 2 hr under high vacuum. The solid was dissolved in hot ethyl acetate. The solution was cooled to room temperature and filtered to remove additional dicyclohexylurea. The filtrate was concentrated under vacuum and the solid residue recrystallized from 90 ml of hot ethanol to yield 42 g (68%): mp 79.5–80°; $[\alpha]^{23}D$ 30.9° (*c* 2, chloroform); ir peaks at 5.7 (active ester), 6.59 and 7.4 (nitro), and 5.9 μ (carbobenzoxy).

Anal. Calcd for $C_{22}H_{24}N_2O_8$: C, 59.45; H, 5.44; N, 6.31. Found: C, 59.42; H, 5.55; N, 6.54.

α-*t*-Butyl-L-aspartic Acid *p*-Nitrophenyl Ester Hydrochloride (III).—To a prehydrogenated suspension of 100 mg of 10% palladium on charcoal in 60 ml of absolute methanol, 2.3 g (5.18 mmol) of II and 1.46 ml of absolute methanol containing 190 mg (5.22 mmol) of hydrogen chloride were added. Hydrogenation was continued for 5 min, at which time the measured amount of hydrogen absorbed was only one half the theoretical amount (57 ml). The reaction mixture was filtered and the filtrate was concentrated to 2 ml under reduced pressure. Anhydrous ether was added to the turbidity point. After standing overnight at −7°, the crystalline product was filtered, washed with anhydrous ether, and dried under vacuum to yield 1.7 g (94.2%), mp 139° dec. Recrystallization from methanol–ether raised the melting point to 146° dec and yielded 1.6 g (90%), $[\alpha]^{23}D$ 14° (*c* 1.37, dimethylformamide). The infrared spectrum showed peaks at 5.7 (active ester), 6.55 and 7.4 μ (nitro); the carbobenzoxy peak was absent at 5.9 μ.

Anal. Calcd for $C_{14}H_{19}ClN_2O_6$: C, 48.48; H, 5.52; Cl, 10.24; N, 8.09. Found: C, 48.76; H, 5.46; Cl, 10.85; N, 8.51.

Poly-α-*t*-butyl-L-aspartate (IV).—A mixture of 1.3 g (3.67 mmol) of III, 3 ml of purified dimethylformamide,[13] and 0.51 ml (3.67 mmol) of purified triethylamine[13] was shaken for 2 days at room temperature. The reaction mixture was diluted with 500 ml of ether and filtered. The solid residue was washed with water to remove triethylamine hydrochloride, with methanol to remove the low molecular weight polymer (29.3%), and finally with ether. The product was dried for 16 hr at 60° (0.1 mm) to yield 116 mg (17%). The infrared spectrum showed peaks at 6.0 (amide I), 6.5 (amide II), and 11.7 μ (*t*-butyl). The molecular weight was 17,000 as determined by the sedimentation equilibrium method.[7] Measurements were made using concentrations in the range of 0.2–0.5% in dimethylacetamide at 26° and a Schieren angle of 75°.

Anal. Calcd for $(C_8H_{13}NO_3)_n$: C, 56.12; H, 7.65; N, 8.18. Found: C, 55.43; H, 7.67; N, 8.26.

(12) This compound was prepared in this laboratory by U. R. Ghatak and G. N. Schmit through the esterification of N-carbobenzoxy-β-methyl-L-aspartic acid with isobutylene in dichloromethane solution in presence of a catalytic amount of sulfuric acid. Selective alkaline hydrolysis of the methyl ester group in aqueous dioxane solution gave N-carbobenzoxy-α-*t*-butyl-L-aspartic acid (I) as an oil. The dicyclohexylamine salt of I, mp 119–120° (from aqueous methanol), gave the correct elemental analysis.

(13) Dimethylformamide and triethylamine were treated with 2% N-carbobenzoxyglycine *p*-nitrophenyl ester overnight, filtered, and distilled to remove primary and secondary amines.

Poly-β-L-aspartic Acid (V).—A solution of 145 mg (0.55 mmol) of IV in 2.91 ml of 90% aqueous trifluoroacetic acid was allowed to stand at room temperature for 50 min. The reaction mixture was diluted with 52 ml of anhydrous ether. The precipitate was centrifuged and the supernatant liquid was decanted. The ether washing was repeated three times and the polymer was dried under vacuum to yield 69.3 mg (73%). The polypeptide was dissolved in 5 ml of water and dialyzed against 800 ml of water for 24 hr. The solution was lyophilized and polymer V (36.3 mg, 48%) was recovered. Lack of absorption at 11.7 μ in the infrared spectrum indicated complete removal of the *t*-butyl groups. The molecular weight was 8000 as determined by the sedimentation equilibrium method. The concentrations used were in the range of 0.2–0.5% in 0.1 *M* aqueous lithium chloride at 26° and a Schieren angle of 75°. When water was used as a solvent for polymer V, a molecular weight of 4500 was obtained. This low value was ascribed to a charge effect which was minimized by using 0.1 *M* aqueous lithium chloride as the solvent.

Anal. Calcd for $(C_4H_5NO_3 \cdot {}^1/_2H_2O)_n$: C, 38.71; H, 4.87; N, 11.30. Found: C, 38.26; H, 5.01; N, 11.20.

Glycine *p*-Nitrophenyl Ester Hydrochloride.—A suspension of 1.21 g (3.67 mmol) of N-carbobenzoxyglycine *p*-nitrophenyl ester[10] was hydrogenated within 2 min using the method described above to yield 0.67 g (78.7%), mp 183.5 dec. Recrystallization from methanol–ether did not change the melting point.

Anal. Calcd for $C_8H_9ClN_2O_4$: C, 41.31; H, 3.90; Cl, 15.24; N, 12.04. Found: C, 41.15; H, 3.87; Cl, 15.55; N, 11.80.

Glycine *p*-Aminophenyl Ester Dihydrochloride.—To a prehydrogenated suspension of 100 mg of 10% palladium on charcoal in 2 ml of glacial acetic acid and 80 ml of absolute methanol, 0.4 g (1.21 mmol) of N-carbobenzoxyglycine *p*-nitrophenyl ester, and 1.12 ml of absolute methanol containing 178 mg (4.87 mmol) of hydrogen chloride were added. Hydrogenation was continued for 5 min, during which time an apparent volume of 81.4 ml of hydrogen was absorbed. The reaction mixture was filtered and the filtrate was concentrated to 2 ml under reduced pressure. Anhydrous ether was added to the solution until the turbidity point was reached. The product, which crystallized on standing overnight at −10°, was filtered, washed with anhydrous ether, and dried under vacuum at 78° to yield 0.211 g (74%), mp 237° dec. The absorptions of the carbobenzoxy group at 5.9 and of the nitro group at 6.4 and 7.4 μ were absent in the infrared spectrum.

Anal. Calcd for $C_8H_{12}Cl_2N_2O_2$: C, 40.05; H, 5.38; Cl, 29.56; N, 11.68. Found: C, 39.86; H, 5.18; Cl, 29.60; N, 11.41.

L-Phenylalanine *p*-Nitrophenyl Ester Hydrochloride.—N-Carbobenzoxy-L-phenylalanine *p*-nitrophenyl ester[11] (0.76 g, 1.81 mmol) was hydrogenated in 2 min as described above to yield 0.47 g (80%), mp 187° dec (recrystallization from methanol–ether did not change the melting point), $[\alpha]^{23}D$ 47.0° (*c* 0.995, methanol).

Anal. Calcd for $C_{15}H_{15}ClN_2O_4$: C, 55.82; H, 4.69; Cl, 10.99; N, 8.68. Found: C, 55.50; H, 4.74; Cl, 11.00; N, 8.71.

α-*t*-Butyl-L-glutamic Acid *p*-Nitrophenyl Ester Hydrochloride.—A methanolic suspension of 1.68 g (3.67 mmol) of N-carbobenzoxy-α-*t*-butyl-L-glutamic acid *p*-nitrophenyl ester was hydrogenated, as above, until half of the theoretical amount of hydrogen had been absorbed to yield 1.04 g (77.7%), mp 128° dec. (recrystallization from methanol–ether did not change the melting point), $[\alpha]^{23}D$ 2.3° (*c* 1.89, methanol).

Anal. Calcd for $C_{18}H_{21}ClN_2O_6$: C, 49.93; H, 5.87; Cl, 9.83; N, 7.78. Found: C, 49.79; H, 5.54; Cl, 9.83; N, 7.91.

N-Carbobenzoxy-L-phenylalanylglycine *p*-Nitrophenyl Ester.—N-Carbobenzoxy-L-phenylalanine (0.597 g, 2 mmol) was dissolved in 20 ml of ethyl acetate and the solution was cooled to −20°. N-Methylmorpholine (0.22 ml, 2 mmol) and isobutylchloroformate (0.28 ml, 2.1 mmol) were added consecutively. The mixture was stirred at −20° for 15 min and 0.466 g (2 mmol) of glycine *p*-nitrophenyl ester hydrochloride and 0.28 ml (2 mmol) of triethylamine were also added consecutively and stirring was continued for 1 hr at −20°. The mixture was stored for 2 hr at −10°. The reaction mixture was concentrated under vacuum and then distributed between 80 ml of chloroform and 30 ml of water. The chloroform layer was washed with 30 ml of water, 20 ml of 0.25 *N* sodium bicarbonate, 30 ml of water, 25 ml of 0.5 *N* hydrochloric acid, and three times with 30 ml of water. The solution was dried over sodium sulfate, filtered, and concentrated under reduced pressure. The product was crystallized from absolute ethanol to yield 0.701 g (73.5%): mp 182–183°; $[\alpha]^{23}D$ −19.8° (*c* 1, dimethylformamide); ir peaks at 5.7 (active ester), 6.0 (amide I), 6.45 (amide II), and 7.4 μ (nitro group).

Anal. Calcd for $C_{25}H_{23}N_3O_7$: C, 62.89; H, 4.86; N, 8.80. Found: C, 62.80; H, 4.95; N, 8.71.

N-Carbobenzoxy-L-phenylalanylglycine *p*-nitrophenyl ester was also prepared using glycine *p*-nitrophenyl ester hydrobromide to yield 2.84 g (77.76%): mp 182–183°, $[\alpha]^{23}D$ −19.2° (*c* 1.92, dimethylformamide).

L-Phenylalanylglycine *p*-Nitrophenyl Ester Hydrochloride.—A suspension of 0.3 g (0.63 mmol) of N-carbobenzoxy-L-phenylalanylglycine *p*-nitrophenyl ester was hydrogenated within 2 min using methanol as described above to yield 0.2 g (83.9%), mp 183.5–184.5° dec. (recrystallization from methanol–ether did not change the melting point), $[\alpha]^{23}D$ 19.6° (*c* 1, methanol).

Anal. Calcd for $C_{17}H_{18}ClN_3O_5$: C, 53.76; H, 4.78; Cl, 9.34; N, 11.06. Found: C, 53.66; H, 5.10; Cl, 9.23; N, 10.52.

Registry No.—II, 6997-15-5; III, 16336-34-8; glycine *p*-nitrophenyl ester hydrochloride, 16336-29-1; glycine *p*-aminophenyl ester dihydrochloride, 16336-30-4; L-phenylalanine *p*-nitrophenyl ester hydrochloride, 16336-31-5; α-*t*-butyl-L-glutamic acid *p*-nitrophenyl ester HCl, 16336-35-9; N-carbobenzoxy-L-phenylalanylglycine *p*-nitrophenyl ester, 16336-36-0; L-phenylalanylglycine *p*-nitrophenyl ester hydrochloride, 16336-32-6.

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