Catalytic Transfer Hydrogenolysis of Protected Peptides using Hydrazine

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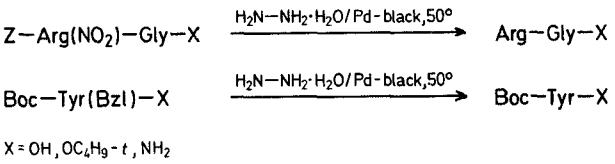
The use of catalytic transfer hydrogenation employing cyclohexene in presence of palladium-black for the removal of some commonly used protecting groups like benzoxycarbonyl, benzyl ester, benzyl ether, and nitro from peptides was reported by us recently[1]. Cyclohexene in the presence of palladium-black (generated *in situ*) is also suitable for the cleavage of a peptide attached to a polystyrene resin through a benzyl ester linkage[2]. The present report concerns some investigations carried out using hydrazine, instead of cyclohexene, as a hydrogen donor in the catalytic transfer hydrogenation of protected peptides. That hydrazine can reduce aromatic nitro compounds in the presence of palladium catalysts is well known[3]. Our investigations demonstrate that it can also be used for the removal of benzoxycarbonyl and nitro groups in protected peptides; indeed, hydrazine appears to be superior to cyclohexene when a nitro group has to be cleaved from a protected peptide. The nucleophilicity of this reagent, however, precludes its use when methyl, ethyl, or benzyl ester groups are present in the protected peptide, but it can successfully be used when protecting groups derived from *t*-butanol are employed. A few typical examples are cited in the Table.

The utility of this method has been demonstrated during the synthesis of two tetrapeptides, Z-Val-Gln-Trp-Leu-OC_4H_9-*t* (corresponding to positions 23–26 of glucagon) and Boc-Tyr(Bzl)-Ser-Phe-Leu-OC_4H_9-*t* (corresponding to positions 51–60 of human growth hormone). Both the sequences were assembled stepwise from the C-terminal end by conventional methods with the modification that the benzoxycarbonyl group after the introduction of each amino acid was removed by hydrazine in presence of palladium-black. In all cases hydrogenolysis was effected at 50°, and apparently this temperature had no deleterious effect in the experiments cited.



The removal of a nitro group from protected peptides is particularly easy. Though the benzyl ether group of Tyr(Bzl) could be easily removed, its removal from Ser(Bzl) was more difficult. The progress of hydrogenolysis could be followed by T.L.C. on silica gel G plates. Hydrazine itself gives a wide yellow spot with ninhydrin which sometimes masks the spot due to the deprotected peptide. In such cases a better way to monitor the completion of the reaction is to look for the absence of the spot due to the protected peptide as this is more easily done.

General Procedure for Hydrogenolysis Using Hydrazine:
A solution of the protected peptide in methanol or ethanol containing palladium-black (one-tenth the weight of peptide) is stirred at 50° and hydrazine hydrate (99–100%; 6 to 8 molar equivalents) is added. After the hydrogenolysis is complete, the catalyst is removed by filtration and the filtrate evaporated to dryness. To remove the excess of hydrazine hydrate, the product may be evacuated over concentrated sulphuric acid or taken up in ethyl acetate or chloroform, and washed with water.

Leu-OC_4H_9-*t*·HCl:
Hydrazine hydrate (99–100%, 2.5 ml) is introduced into a stirred mixture of Z-Leu-OC_4H_9-*t* (2.24 g), methanol (4 ml), and palladium-black (0.2 g) maintained at 50°. After the completion of the reaction (~1.5 h), the catalyst is filtered off, washed with methanol, and the combined washings evaporated in vacuo at 40° to an oil. This oil is extracted into ethyl acetate (30 ml), and washed with water (2 × 10 ml), dried with sodium sulphate, and evaporated. The resultant oil is dissolved in dry ether (100 ml), mixed with 2 normal hydrochloric acid in methanol (3.5 ml), and stored in the cold for 0.5 h when Leu-OC_4H_9-*t*·HCl crystallises as long needles; yield: 1.41 g (90%); m.p. 172–173°, $[\alpha]_D^{25}$: +12.0° (*c* 2, C_2H_5OH); Lit.[4], m.p. 166–167°; $[\alpha]_D^{25}$: +12.4° (*c* 2, C_2H_5OH).

Z-Ser-Phe-Leu-OC_4H_9-*t*:
A mixture of Z-Phe-Leu-OC_4H_9-*t* (0.5 g), palladium-black (0.05 g), ethanol (4 ml), and hydrazine hydrate (99–100%; 0.5 ml) is warmed with stirring to 50°. After the completion of the hydrogenolysis (0.5 h), the catalyst is removed by filtration. Evaporation of the filtrate furnished an oil which is dissolved in ethyl acetate (20 ml), and washed with water (2 × 10 ml), dried with sodium sulphate, and evaporated in vacuo to yield Phe-Leu-OC_4H_9-*t*; yield: 0.34 g (95%), as an oil which is then condensed with Z-Ser (0.24 g) by the dicyclohexylcarbodiimide method to furnish Z-Ser-Phe-Leu-OC_4H_9-*t*; yield: 0.45 g (80%); m.p. 160–161°; $[\alpha]_D^{25}$: −38.46° (*c* 1.17, CH_3OH); Lit.[7], m.p. 160–161°; $[\alpha]_D^{25}$: −39.2° (*c* 1, CH_3OH).

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Table. Deprotection of Peptides

Substrate	Reaction Time [h]	Product	Yield [%]	m.p.	Specific Rotation	Lit. m.p.	Lit. Specific Rotation
Z-Leu-OC_4H_9-*t*	1.5	Leu-OC_4H_9-*t*·HCl[4]	90	172–173°	+12.0° (*c* 2, C_2H_5OH)	172–173°	+12.4° (*c*, 2, C_2H_5OH)
Boc-Tyr(Bzl)	1.0	Boc-Tyr[4]	96	135–137°	+5.9° (*c* 2, AcOH)	136–138°	+5.9° (*c* 2.04, AcOH)
Boc-Ser(Bzl)	8.0	Boc-Ser·H_2O[4]	60	76–78°	−4.5° (*c* 1, AcOH)	75–78°	−4.6° (*c* 1, AcOH)
Boc-Arg(NO_2)-Leu-OC_4H_9-*t*	1.0	Arg-Leu·2TFA[5]	90	200–202°	+9.1° (*c* 1, H_2O)	203–204°	+9.1° (*c* 1, H_2O)
Z-Arg(NO_2)-Gly-NH_2	0.5	Arg-Gly-NH_2·dipicrate[6]	95	209–210°	+15.8° (*c* 1, 50% aq. acetone)	210–210.5°	+15.9° (*c* 1, 50% aq. acetone)
Z-Phe-Leu-OC_4H_9-*t*[7]	0.5	Phe-Leu-OC_4H_9-*t*[a]	95	gum	—		
Z-Ser-Phe-Leu-OC_4H_9-*t*[7]	0.5	Ser-Phe-Leu-OC_4H_9-*t*[a]	95	gum	—		
Z-Trp-Leu-OC_4H_9-*t*	1.5	Trp-Leu-OC_4H_9-*t*·AcOH[8]	95	106–108°	−18.9° (*c* 1.3, CH_3OH)	105–107°	−18.51° (*c* 1.3, CH_3OH)
Z-Gln-Trp-Leu-OC_4H_9-*t*	4.0	Gln-Trp-Leu-OC_4H_9-*t*[8]	90	134–136°	−21.6° (*c* 1.5, CH_3OH)	134–136°	−21.74° (*c* 1.5, CH_3OH)
Z-Val-Gln-Trp-Leu-OC_4H_9-*t*	1.5	Val-Gln-Trp-Leu-OC_4H_9-*t*[8]	95	188–189°	−44.5° (*c* 1.1, C_2H_5OH)	188–189°	−44.0° (*c* 1.1, C_2H_5OH)

[a] These free bases were directly used for the preparation of the peptides, Z-Ser-Phe-Leu-OC_4H_9-*t* and Boc-Tyr(Bzl)-Ser-Phe-Leu-OC_4H_9-*t*, which were identified by comparing their m.p. and specific rotations with those reported in literature[7].

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