An Advantageous Method for the Rapid Removal of Hydrogenolysable Protecting Groups under Ambient Conditions; Synthesis of Leucine-enkephalin

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The facile removal of protecting groups from labile or reactive organic functionalities is an important objective, especially in such strategies as minimal protection peptide synthesis where mild, efficient techniques are essential. Among desirable characteristics of any deprotection scheme are the following: (1) low cost, (2) rapidity, (3) selectivity, (4) mild conditions, usually avoiding strong acid or base, and (5) broad applicability. Recently, a number of improvements in the area of catalytic transfer hydrogenation (CTH) have been reported[1-5] which greatly simplify these procedures and some even reduce the operating conditions to standard room temperature and pressure[3,4,5]. These employed such CTH agents as cyclohexene[1], hydrazine[2], cyclohexadiene[3], and formic acid[4,5]. We report a further refinement of the CTH system which now appears to achieve all the above objectives, and which may find utility in automated or continuous flow applications.

Since we believe the active species in formic acid transfer hydrogenation to be the formate anion, we developed the present system which utilizes ammonium formate as the hydrogen donor and palladium-on-carbon as the heterogeneous catalyst. Using methanol or dimethylformamide, a variety of amino acids and peptide derivatives were smoothly deprotected in minutes as shown in the Table. The protecting groups removed included the benzyloxycarbonyl, benzyl ether, nitro, and benzyl ester; in view of the neutral conditions employed, such common but acid-labile amine protecting groups as the *t*-butyloxycarbonyl (Boc) group were unaffected by this treatment. The use of 10% Pd—C/ammonium formate makes this a rapid, low-cost alternative to palladium black and reduces the work-up to a simple filtration and (optional) extraction/lyophilization operation. Most of the reactions are complete in *less than one minute* as monitored by the disappearance of starting material and concomitant formation of product via reversed-phase high-pressure liquid chromatography (H.P.L.C.) methods. The arginine N^g-nitro group was cleanly removed *in less than five minutes,* while benzyl ethers (serine and threonine) were deprotected in 30 and 90 minutes, respectively, although in the latter case an acidic medium was required for the reactions to proceed to completion.

A quantitative comparison of this method with several other alternatives is graphically depicted in the Figure. The percent relative deprotection values were obtained by integrating starting reactant and product peaks for a standard Z-Phe-Leu-OC_4H_9-*t* sample on reversed phase H.P.L.C. Interestingly, no deprotected material could be detected (by H.P.L.C.) even after 10 minutes when Z-L-Phe-L-Leu-OC_4H_9-*t* was subjected to formic acid CTH with 10% palladium-on-carbon, while under identical conditions, ammonium formate completely removed the benzyloxycarbonyl group in less than 1 minute. Even with the far more reactive (and expensive) freshly prepared palladium black catalyst[6], the deprotection with formic acid is no more rapid than with ammonium formate/10% palladium-on-carbon.

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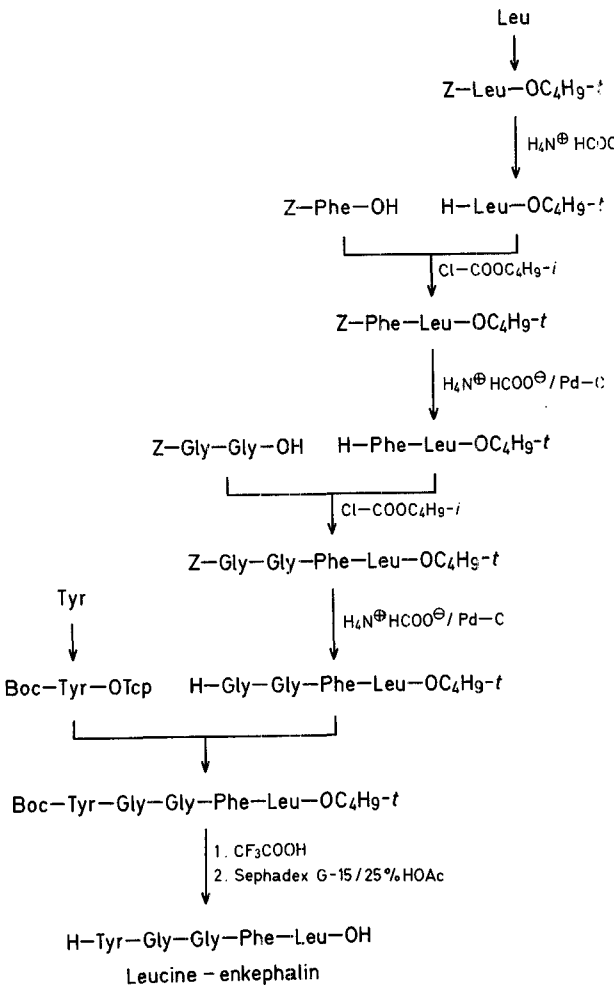

Z = benzyloxycarbonyl
OTcp = 2,4,5-trichlorophenyl ester

Scheme A: Synthesis of Leucine-enkephalin by solution methods using ammonium formate/palladium-on-carbon for benzyloxycarbonyl deprotection at room temperature and pressure

As a racemization test, the model compounds Z-L-Phe-L-Leu-OC_4H_9-*t* and Z-D-Phe-L-Leu-OC_4H_9-*t* were prepared and subjected to benzyloxycarbonyl removal by ammonium formate and 10% palladium-on-carbon. H.P.L.C. analyses of the reaction mixtures showed no contamination (<0.25%) by diastereomeric by-products (L-Phe-L-Leu-OC_4H_9-*t* and D-Phe-L-Leu-OC_4H_9-*t* have retention times of 7.94 and 9.78 minutes, respectively, on a C-18 reversed phase Zorbax analytical column with a 45–60% methanol/0.25 molar ammonium acetate (pH 4.1) gradient over 15 minutes).

To further assess the utility of this method, the biologically active peptide, leucine-enkephalin, was synthesized using ammonium formate/10% palladium-on-carbon deprotection for intermediary benzyloxycarbonyl-protected precursors, as indicated in Scheme A. The final amino acid was incorporated as its *N*-α-*t*-Boc derivative and both *t*-butyl-based protecting groups were removed by trifluoroacetic acid treatment, the only acid exposure to which the peptide was subjected. Following a single gel filtration, a remarkably homogeneous product was obtained which corresponded in all respects, including H.P.L.C. retention times and optical rotation, to a highly purified authentic leucine-enkephalin sample prepared via standard conditions of solid-phase peptide synthesis. The peptide was evaluated for its biological potency by the response of the C57 Black 6 mouse strain to the standard hot plate test and it proved fully as active as an authentic sample.

The utility of ammonium formate is apparently related to formate anion as a facile hydrogen donor. Other agents including sodium formate[8] as well as a more lipophilic dicyclohexylammonium formate have also proven efficacious; the latter might be expected to provide solubility advantages in some applications[9]. A more detailed analysis of the use of these reagents and a comparison with other hydrogenation alternatives will be presented in a full report.

H.P.L.C. analyses were performed with a C-18 reversed-phase Zorbar column using a 0.25 molar ammonium formate/methanol gradient (45–60%). T.L.C. analyses were carried out on Merck F 254 silica gel plates with propanol/water (2:1); butanol/acetic acid/water (4:1:1); chloroform/acetic acid (95:5).

General Procedure for Hydrogenolysis Using Ammonium Formate:

A solution of the protected peptide in methanol or dimethylformamide or a mixture of methanol and acetic acid containing 10% palladium-on-carbon (1/10 to 1/2 the weight of the peptide) and ammonium formate (2 to 4 equivalents) in a test tube is allowed to stand (or is shaken on a vortex mixer) at ambient temperature for 5 to 10 min. After the hydrogenolysis is complete (as monitored by T.L.C. or H.P.L.C.), the catalyst is removed by filtration through celite and the filtrate evaporated to dryness. To remove excess ammonium formate, the reaction product is either lyophilized or dissolved in an organic solvent and washed with saturated sodium chloride solution.

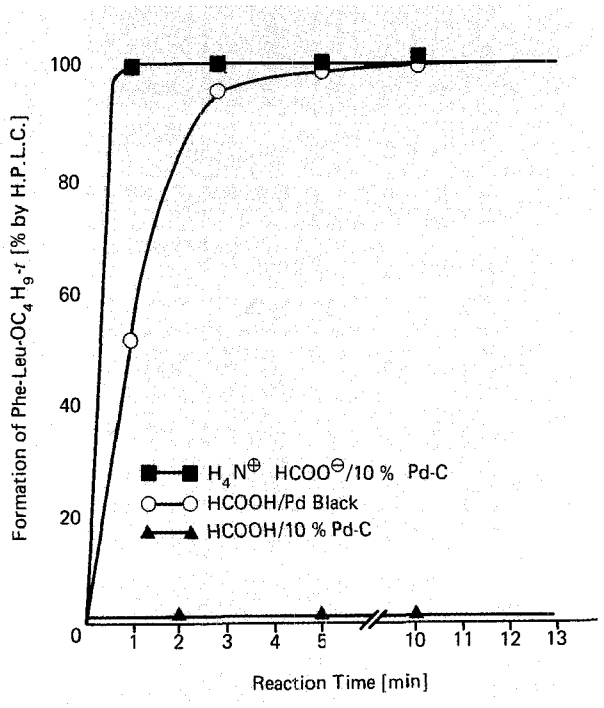

Figure. Relative Rates of Removal of the Benzyloxycarbonyl Group From from Z-Phe-Leu-OC_4H_9-*t* as monitored by H.P.L.C. (values are corrected percentages based on standard samples of protected and deprotected materials and based on relative absorbances at 254 nm)

Table. Ammonium Formate/10% Palladium-on-Carbon Deprotection

Substrate	Product	Reaction time [min]	Yield [%][a]	m.p. [°C] found	m.p. [°C] reported	$[\alpha]_D$ Specific Rotation[b] found	$[\alpha]_D$ Specific Rotation[b] reported	Ref.
Boc-Asp(β-OBzl)	Boc-Asp·2[(*c*-$C_6H_{11})_2NH_2^{\oplus}$]	3	98	176–177°	176–177°	+10.9° (*c* 1.05, CH_3OH)	+10.9° (*c* 1, CH_3OH)	10
Boc-Glu-(γ-OBzl)	Boc-Glu·2[(*c*-$C_6H_{11})_2NH_2^{\oplus}$]	3	81	172–173°	171–172°	+9.1° (*c* 1.05, CH_3OH)	+9.1° (*c* 1, CH_3OH)	10
Boc-Tyr(Bzl)	Boc-Tyr	10	85	136–137°	136–138°	+3.01° (*c* 2.09, CH_3OH)	+3.9° (*c* 2.04, AcOH)	10
Boc-Ser(Bzl)[c]	Boc-Ser·[(*c*-$C_6H_{11})_2NH_2^{\oplus}$]	30	70	141–142°	140–142°	+13.5° (*c* 3.26, CH_3OH)	+13.3° (*c* 3.06, CH_3OH)	10
Boc-Thr(Bzl)[c]	Boc-Thr·[(*c*-$C_6H_{11})_2NH_2^{\oplus}$]	90	90	153–154°	154–155°	+11.2° (*c* 1.01, CH_3OH)	+11.37° (*c* 0.99, CH_3OH)	10
N^{α}-Boc-Lys(ε-Z)	Boc-Lys	5	95	202–204°	203–204°	−10.8° (*c* 0.9, AcOH)	−10.7° (*c* 0.87, AcOH)	10
Z-Phe	Phe	5	95	n. d.	270–275° (dec)	−34.7° (*c* 0.7, H_2O)	−34.4° (*c* 2, H_2O)	11
Z-Trp	Trp	5	95	n. d.	280–285° (dec)	−31.8° (*c* 1.09, H_2O)	−32 ± 0.5° (*c* 1, H_2O)	11
Z-Phenylalaninol (*N*-Z-2-amino-3-phenyl-1-propanol)	L-Phenylalaninol (2-amino-3-phenyl-1-propanol)	5	98	91–92°	92–94°	−23.0° (*c* 1.2, 1 normal HCl)	−22.8° (*c* 1.2, 1 normal HCl)	12
Boc-Arg(N^g-NO_2)-Leu-OC_4H_9-*t*	Arg-Leu·2CF_3COOH	5	89	200–202°	203–204°	+9.1° (*c* 1.1, H_2O)	+9.0° (*c* 1.5, H_2O)	13
Z-Trp-Leu-OC_4H_9-*t*	Trp-Leu-OC_4H_9-*t*·AcOH	5	98	106–108°	105–107°	−18.5° (*c* 1.3, CH_3OH)	−18.5° (*c* 1.3, CH_3OH)	14
Z-Leu-OC_4H_9-*t*	Leu-OC_4H_9-*t*	5	98	oil		+19.1° (*c* 2, C_2H_5OH)	+21.6° (*c* 2.5, C_2H_5OH)	16
Z-Phe-Leu-OC_4H_9-*t*	Phe-Leu-OC_4H_9-*t*	5	98	oil		—[d]	—	15
Z-Gly-Gly-Phe-Leu-OC_4H_9-*t*	Gly-Gly-Phe-Leu-OC_4H_9-*t*·AcOH	5	92	—[e]		−19.3° (*c* 0.98, CH_3OH)[f]	—	—

[a] Refers to the actual isolated yields; no attempts were made to maximize yields.
[b] Optical rotations were determined on a Perkin-Elmer 241MC Polarimeter between 20–25 °C, coincident with the Lit. reported temperatures.
[c] The reaction medium was a 1:1 mixture of CH_3OH/AcOH.
[d] Identified as Z-Gly-Gly-Phe-Leu-OC_4H_9-*t*, see experimental.
[e] The m.p. could not be determined accurately because of the hygroscopic nature of the compounds.
[f] This was characterized by conversion to leucine-enkephalin, see experimental.

N-α-t-Boc-aspartic Acid Bis[dicyclohexylammonium] Salt:
To a solution of Boc-Asp(β-OBzl) (520 mg, 1.61 mmol) in methanol (5 ml) containing ammonium formate (300 mg), 10% palladium-on-carbon (300 mg) is added and the mixture allowed to stand for 2 min. The reaction mixture is then filtered through celite and the filtrate evaporated to dryness. The residue is extracted into ethyl acetate (2 × 25 ml), the extract washed with saturated sodium chloride (2 × 10 ml), dried with sodium sulfate, and concentrated in vacuo to a small volume; subsequently, dicyclohexylamine (0.70 ml, 3.52 mmol) in tetrahydrofuran (2 ml) is added to form the bis-dicyclohexylammonium salt of Boc-aspartic acid. The crystalline salt is filtered and washed with dry ether to give the product; yield: 940 mg (98%); m.p. 175–177 °C; $[\alpha]_D^{25}$: +10.9° (*c* 1.05, CH_3OH); Lit.[10], m.p. 176–177 °C; $[\alpha]_D^{25}$: +10.9° (*c* 1, CH_3OH).

Leucine-enkephalin:
Z-Gly-Gly-Phe-Leu-OC_4H_9-t: 10% Palladium-on-carbon (100 mg) is introduced into a solution of Z-Phe-Leu-OC_4H_9-*t* (472 mg, 1.01 mol, obtained according to Lit.[15]) and ammonium formate (250 mg, 3.97 mmol) in methanol (5 ml), and the mixture is allowed to stand for 5 min. After completion of the hydrogenolysis (monitored by T.L.C.), the mixture is filtered through celite and the catalyst washed with methanol (2 × 10 ml). The combined washings and filtrate are evaporated in vacuo at room temperature to an oil, which is extracted into ethyl acetate (2 × 25 ml), the extract is washed with saturated sodium chloride solution (2 × 10 ml), and dried with sodium sulfate. The solvent is evaporated in vacuo to furnish *Phe-Leu-OC_4H_9-t* as an oil (pure by T.L.C.); yield: 318 mg (95%).

Phe-Leu-OC_4H_9-*t* (318 mg, 0.95 mmol) is coupled with Z-Gly-Gly[17] (254 mg, 0.95 mmol, obtained from Vega Biochemicals) using 2-methylpropyl carbonochloridate (0.12 ml, 0.95 mmol) – mixed anhydride method according to Lit.[18] – to give *Z-Gly-Gly-Phe-Leu-OC_4H_9-t*; yield: 333 mg (60%); m.p. 147–148 °C; $[\alpha]_D^{20}$: −13.3° (*c* 0.98, CH_3OH).

$C_{31}H_{42}N_4O_7$	calc.	C 63.90	H 7.27	N 9.62
(582.7)	found	64.03	7.25	9.59

Boc-Tyr-Gly-Gly-Phe-Leu-OC_4H_9-t: Z-Gly-Gly-Phe-Leu-OC_4H_9-*t* (530 mg, 0.91 mmol) is deprotected with 10% palladium-on-carbon (50 mg) and ammonium formate (300 mg) in methanol (10 ml) as described above to give *AcOH-Gly-Phe-Leu-OC_4H_9-t*; yield: 425 mg (92%); $[\alpha]_D^{20}$: −19.3° (*c* 0.98, CH_3OH).

AcOH-Gly-Gly-Phe-Leu-OC_4H_9-*t* (200 mg, 0.39 mmol) is coupled with Boc-Tyr-OTcp (253 mg, 0.55 mmol, obtained according to Lit.[19]) using hydroxybenzotriazole (84 mg, 0.55 mmol) in dimethylformamide (2 ml) to give *Boc-Tyr-Gly-Gly-Phe-Leu-OC_4H_9-t*; yield: 266 mg (95%); m.p. 107–108 °C; $[\alpha]_D^{20}$: −17.0° (*c* 1, DMF); Amino Acid Analysis: Tyr 1.0 (1); Gly 1.9 (2); Phe 1.1 (1); Leu 1.0 (1).

Leucine-enkephalin: Boc-Tyr-Gly-Gly-Phe-Leu-OC_4H_9-*t* (160 mg, 0.23 mmol) is deprotected by treatment with 50% trifluoroacetic acid containing 10% anisole in dichloromethane for *1 h to give leucine-enkephalin;* yield: 133 mg (88%); m.p. 155–158 °C; $[\alpha]_D^{25}$: +25.7° (*c* 1.34, 95% AcOH); Lit.[20], m.p. 157–161 °C; $[\alpha]_D^{20}$: +26.4° (*c* 1, 95% AcOH).

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