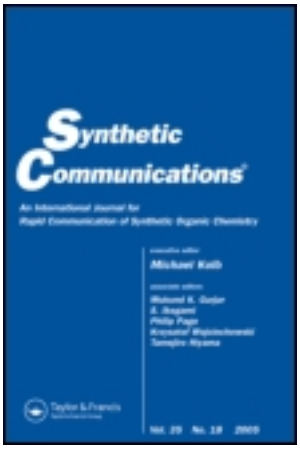

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MW-Enhanced High-Speed Deprotection of Boc Group Using p-TsOH and Concommitant Formation of N-Me-Amino Acid Benzyl Ester p-TsOH Salts

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MW-Enhanced High-Speed Deprotection of Boc Group Using *p*-TsOH and Concommitant Formation of *N*-Me-Amino Acid Benzyl Ester *p*-TsOH Salts

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Abstract: A high-speed, complete deprotection of Boc group from Boc amino acids and protected peptide esters employing *p*-TsOH in toluene under microwave irradiation is found to be complete in 30 s. The deprotection can be carried out in methanol and acetonitrile also. Under the present conditions, C-peptide benzyl esters and O-benzyl ethers have been found to be stable. This has permitted us to carry out the synthesis of [Leu]enkephalin employing the Boc/Bzl-group strategy. Further more, it has been found that both N^{α}-Fmoc and N^{α}-*Z* groups are completely stable. The present conditions can be extended for the concomitant removal of the Boc group and the formation of C-benzyl amino acid esters as well. This has been utilized for the synthesis of *N*-Me amino acid benzyl esters starting from Boc-*N*-Me amino acids in a single step.

Keywords: Boc group, deprotection, microwave irradiation, *N*-Me amino acid benzyl esters

INTRODUCTION

For the chemical synthesis of peptides and small proteins in solution, the *tert*-butoxycarbonyl (Boc) group is the preferred urethane-type-protecting group

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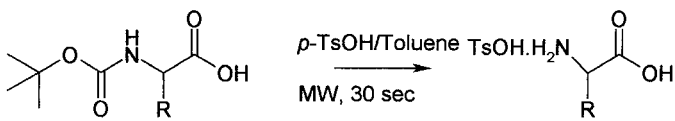
for N^{α}-amino protection of amino acids over an equally popular benzyloxycarbonyl (**Z**) group. This is primarily because it is cleaved by relatively mild acids. Consequently, it is one of the most commonly employed amino-protecting groups in solution-phase peptide chemistry.[1] There is a necessity for the protection of several functional groups present in the side chains of the amino acids. The stability of such side chain–protecting groups during the elongation of the peptide chain and their removal at the end of the synthesis are the key factors in the selection of protecting groups. This has led to the use of combinations of **Z**/^{t}Bu, Boc/Bzl, and Fmoc/^{t}Bu strategy.[2–4]

In general, the Boc group is deprotected by using HCl/AcOH, dioxane EtoAc, anhydrous liquid HF, HBr/AcOH, 10% H_2SO_4/dioxane, 98% HCO_2H, and so for.[5] The use of Lewis acid $BF_3 \cdot Et_2O$ in AcOH[6] and several silicon reagents such as $SiCl_4$-phenol,[7] TMSCl-phenol,[8] TMSI,[9] TMSOTf-TFA,[10] and TMSOTf-2,6-lutidine[11] have been explored. Boc-group deprotection was performed by using $AlCl_3$ doped with neutral alumina under microwave (MW) irradiation for 1–2.5 min (with 76–93% yield)[12] also. Removal of the Boc group using the strong acid TFA either neat or in combination with CH_2Cl_2 (50%) is commonly preferred and more convenient than deprotection with mineral acids or 98% HCO_2H because, after deprotection, excess of trifluoroacetic acid (TFA) can be easily and completely removed (bp of TFA is 71–72°C). The selective cleavage of the Boc group using an organic acid *p*-toluenesulfonic acid (*p*-TsOH) in ether/ethanol, 1,4-dioxane, AcOH-CH_2Cl_2, or acetonitrile requires several hours.[13–17] Over a period of time, the microwave irradiation technique found several applications in organic synthesis[18–20] In the present communication, we describe the utility for the rapid removal of the Boc group in peptide synthesis using *p*-TsOH.

Initial experiments in the present studies centered on the deprotection of the Boc group from amino acids under MW irradiation. (The microwave reaction was carried out in a LG MS 194A microwave oven producing microwave radiation with a frequency of 2450 MHz. The microwave oven is 1200-W oven and the reaction was specifically carried out at 60% of the total power output, which would correspond to an average power of 720 W.) In a typical procedure, a solution of Boc-amino acid in toluene and *p*-TsOH was exposed to MW irradiation (Scheme 1). The deprotection, as monitored by TLC, was found to be complete in 30 s. Further more, deprotection was confirmed by recording IR spectra (revealed by the absence of a urethane carbonyl stretching vibrational frequency in the region



Scheme 1. Deprotection of Boc group under MW irradiation.

1690–1705 cm^{-1}). After cooling, in most of the cases, the resulting amino acid *p*-TsOH salt separated out as a solid. Filtration, washing with toluene, and, if necessary, recrystallization using suitable solvent resulted in the isolation of the product (Table 1). The deprotection has been found to be complete within 30 s when Boc derivatives were dissolved in acetonitrile and methanol also. Because of their low bp, it was necessary to use a greater quantity of solvent. In addition, the use of toluene led to the separation of the amino acid ester salt as a solid, which can be easily isolated by filtration.

The studies are then extended to demonstrate its utility for the removal of the Boc group in peptide synthesis. It has been found that the use of two equivalents of *p*-TsOH in toluene is sufficient to achieve the complete deprotection under MW irradiation within 30 s (Table 2). The deprotection of the Boc group from Boc-peptide esters when carried out using acetonitrile and MeOH was also found to be smooth and complete. As reported, the deprotection of the Boc group using *p*-TsOH in acetonitrile at ambient temperature requires about 3 to 4 h.[13,14] The deprotection carried out by refluxing the toluene using an oil bath (around 105–110°C) took about 2 h for the complete removal of the Boc group. Thus, employing the present conditions, the removal of the Boc group from several dipeptides as well as all the four Boc-protected intermediates in the synthesis of pentapeptide [Leu]enkephalin have been carried out (Scheme 2). The final pentapeptide was obtained in 63% yield [Yield 63%; mp 158–59°C; found: C, 60.48; H, 6.83; N, 12.40. $C_{28}H_{37}N_5O_7$ calcd. for C, 60.53; H, 6.71; N, 12.60%; $[\alpha]_D^{25}$ −22.9 (c = 1, DMF); ^{1}H NMR (δ, $CDCl_3$): 0.83–0.9 (6H, m), 1.14 (2H, t), 1.6 (lH, m), 2.65 (4H, m), 2.8–3.2 (4H, m), 3.4 (2H, br) 3.6–4.0 (3H, m), 5.7–6.1

Table 1. Physical characteristics for the amino acid derivatives

Entry	Protected amino acid	Product	Time (s)	Mp (°C)	Yield (%)
1	Boc-Ala-OH	TsOH · H-Ala-OH	30	170	96
2	Boc-Val-OH	TsOH · H-Val-OH	30	66–68	95
2	Boc-Phe-OH	TsOH · H-Phe-OH	30	114–16	98
2	Boc-Cys(Bzl)-OH	TsOH · H-Cys(Bzl)-OH	30	156–57	94
3	Boc-Trp-OH	TsOH · H-Trp-OH	45	204–05	96
4	Boc-Glu(OBzl)-OH	TsOH · H-Glu(OBzl)-OH	30	114–16	92
5	Fmoc-Lys(Z)-OH	No reaction[a]	—	—	—
6	Fmoc-Ser(Bzl)-OH	No reaction[a]	—	137–38	—
7	Fmoc-Asp(OBzl)-OH	No reaction[a]	—	120–30	—
8	Fmoc-Arg(Pmc)-OH	No reaction[a]	—	132–33	—
9	*Z-N*Me-Leu-OH	No reaction[a]	—	—	—

[a]MW irradiation for 15 min in 15 cycles with 720 W of power has resulted in no change of the reactants and the starting compounds have been isolated completely. No decomposition was noticed.

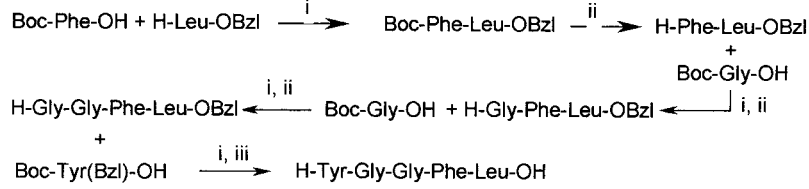
Table 2. List of peptides deportected using *p*-TsOH under MW irradiation

Entry	Protected peptide	Product[a]	Time (s)	Mp (°C)	Yield (%)
1	Boc-His-Pro-OMe	H-His-Pro-OMe	30	145	89
2	Boc-Ile-Gly-OMe	H-Ile-Gly-OMe	30	182–83	92
3	Boc-Phe-Ile-OMe	H-Phe-Ile-OMe	30	102	96
4	Boc-Pro-Phe-OMe	H-Pro-Phe-OMe	30	177–78	88
5	Boc-Val-Tyr-OEt	H-Val-Tyr-OEt	30	113–15	86
6	Boc-Ser-Gly-OBzl	H-Ser-Gly-OBzl	30	170	90
7	Boc-Arg(NO_2)-Ala-OBzl	H-Arg(NO_2)-Ala-OBzl	30	170–71	82
8	Boc-Gly-Leu-Val-OMe	H-Gly-Leu-Val-OMe	30	115–17	88
9	Boc-Val-Tyr-Pro-OBzl	H-Val-Tyr-Pro-OBzl	30	50–60	8
10	Boc-Phe-Leu-OBzl	H-Phe-Leu-OBzl	30	174–76	84
11	Boc-Gly-Phe-Leu-OBzl	H-Gly-Phe-Leu-OBzl	30	168–70	78
12	Boc-Gly-Gly-Phe-Leu-OBzl	H-Gly-Gly-Phe-Leu-OBzl	30	154–56	76

[a]Mass and ^{1}H NMR spectra are satisfactory.

(4H, m), 7.2–7.6 (9H, m), and 8.2 (lH, s). Mass: $(M + H)^+$556.28.]. Its purity, as analyzed by HPLC, was satisfactory. The ^{1}H NMR and mass spectra also confirmed the same.

During this work, a systematic study regarding the stability of various protecting groups normally employed for carboxyl protection of amino acids and side chains of several bifunctional amino acids has been carried out. It is found that amino acid benzyl, methyl, and ethyl esters are completely stable. The exposure of amino acid benzyl ester [Fmoc-Asp(OBzl)-OH] or peptide benzyl ester (H-Phe-Leu-OBzl) to MW irradiation for 15 min in 15 cycles with 720 W of power has resulted in no change of the reactants (as analyzed by TLC as well as by IR). Further more, it has been found that



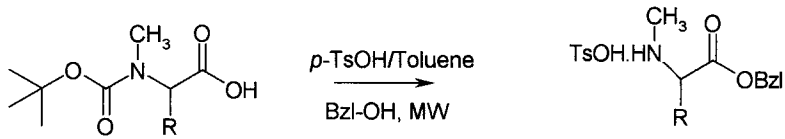
Scheme 2. Synthesis of Leu-enkephalin; (i) coupling employing HBTU using standard conditions, (ii) deprotection of Boc group using *p*-TsOH under MW irradiation, and (iii) CTH using 98% HCOOH/Pd-C/MeOH as solvent.

N^{α}-Fmoc and **Z** groups, Asp(OBzl), Ser(Bzl), and Arg(Pmc) have been found to be completely stable (Table 1).

Our group is half-way to the solution synthesis of cyclosporine **O** and its analogues in few gram quantities by employing Boc chemistry with the segment condensation approach. For this work, we needed to make large quantities of *N*-Me-Leu-OBzl and *N*-Me-Val-OBzl. In general, the preparation of *N*-Me amino acid benzyl esters has been carried out in two steps. In the first step, N^{α}-protected (Boc, **Z**, or Fmoc) *N*- Me amino acids have been converted to methyl or benzyl esters. Then, N^{α}-protecting group was removed to obtain *N*-Me amino acid esters.[21–25] Consequently, we made initially Boc-*N*-Me-Leu-OBzl employing Boc-*N*-Me-Leu-OH, Cs_2CO_3, and benzyl bromide following the reported procedure.[26] After the deprotection of Boc group using TFA from the resulting Boc-*N*-Me-Leu-OBzl, *N*-Me-Leu-OBzl was obtained in 47% yield. Consequently, we attempted to deblock the Boc group using *p*-TsOH under MW irradiation. The deprotection was found to be clean and complete. Based on these results as well as our recently published method for the synthesis of amino acid benzyl ester *p*-TsOH salts,[27] we reasoned that both the deprotection of the Boc group and simultaneous formation of the benzyl ester can be accomplished in a single step (Scheme 3). It is found that the use of two equivalents of *p*-TsOH along with BOC-*N*-Me-Leu-OH and benzyl alcohol in toluene and exposure of the mixture to MW irradiation for 30–45 s has resulted in the direct formation of *N*-Me amino acid benzyl ester *p*-TsOH salt in one pot. Under these conditions, we have prepared few other benzyl esters required for our synthesis (Table 3).

In conclusion, these studies demonstrate that the Boc group can be deprotected very rapidly in solution synthesis of peptides. The use of two equivalents of *p*-TsOH is sufficient for the complete removal of the Boc group from peptides in 30 s. On the other hand, deprotection of the Boc group at ambient temperature needs several hours. After the deprotection, the resulting peptide ester *p*-TsOH salts can be easily converted to the corresponding amino free amino acid ester and employed for the further extension of the chain. In "green chemistry," which aims to reduce the use of toxic chemicals, the use of *p*-TsOH in place of TFA (regularly used in peptide synthesis) may find wide acceptability. The utility of the present procedure for the removal of the Boc group in solid-phase synthesis of [Leu]enkephalinamide is in progress.

Further, the synthesis of *N*-Me amino acid benzyl ester is usually accomplished in a two-step process involving the conversion of Boc or Fmoc-*N*-Me



Scheme 3. Concomitant deprotection of Boc group and formation of *N*-Me amino acid benzylester TsOH salt.

Table 3. *p*-TsOH-included MW-irradiated concomitant deprotection of the Boc group and formation of *N*-Me amino acid benzyl esters

Entry	Boc-*N*-Me amino acid	Product[a]	Time (s)	Mp (°C)	Yield (%)	$[\alpha]_D^{20}$ (c = 1, $CHCl_3$)[24]
1	Boc-*N*-Me-Leu-OH	H-*N*-Me-Leu-OBzl	30	gum	96	−6.98 (−6.90, c = 1, $CHCl_3$)
2	Boc-*N*-Me-D-Leu-OH	H-*N*-Me-D-Leu-OBzl	30	—	95	+6.42 (+6.06, c = 0.90, $CHCl_3$)
3	Boc-*N*-Me-Val-OH	H-*N*-Me-Val-OBzl	30	—	92	−5.13 (−5.20, c = 1.18, $CHCl_3$)
4	Boc-*N*-Me-D-Val-OH	H-*N*-Me-D-Val-OBzl	30	—	93	+5.28 (+5.10, c = 1, $CHCl_3$)

[a]The IR analysis confirms the presence of benzyl ester and the absence of the peak corresponds to urethane carbonyl vibrational stretching. Spectral data is given in the experimental section.

amino acid to the corresponding benzyl ester and then the removal of protecting group. It is now demonstrated that the synthesis of *N*-Me amino acid benzyl esters in a single operation rapidly and with high yields under MW irradiation technique can be accomplished.

EXPERIMENTAL

Melting points were determined by the capillary method and are uncorrected. IR spectra were recorded on a Nicolet model impact 400D FT-IR spectrometer (KBr pellets, 3 cm^{-1} resolution). ^1H and ^{13}C NMR spectra were recorded on a Bruker AMX 400-MHz spectrometer. Mass spectra were recorded on PE-SCIEX 150 EX LCMS. Specific rotations were recorded on a Rudolf Research Autopol IV automatic polarimeter. The TLC analysis was carried out on precoated silica-gel plates using solvent systems (a) ethyl acetate–petroleum ether (35:65 v/v) and (b) chloroform–methanol–acetic acid (40:2:1 v/v). All solvents were freshly distilled prior to use.

General Procedure for Boc-Group Deprotection

A mixture of Boc amino acid or peptide ester (1 mmol) and *p*-TsOH (2 mmol) in toluene (5 mL) in a beaker was exposed to microwave irradiation until the completion of the deprotection. On cooling to rt, the amino acid *p*-TsOH salt has been separated as a solid, which was filtered and washed with toluene (2 mL).

In the case of peptides, after the deprotection of the Boc group, the product was isolated as follows: the resulting peptide ester *p*-TsOH salt was

dissolved in water (10 mL) and the organic layer was separated. The aqueous layer was neutralized using 10% Na_2CO_3 and extracted with ethyl acetate (3×10 mL). The combined organic layer was given a water wash (1×10 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to obtain the amino free peptide ester.

General Procedure for the Preparation of *N*-Me Amino Acid Benzyl Ester Starting from Boc-*N*-Me Amino Acid

A mixture of Boc-*N*-Me amino acid (1 mmol), *p*-TsOH (2 mmol), benzyl alcohol (5 mmol), and toluene (5 mL) was exposed to microwave irradiation. After completion of the reaction, the resulting mass was cooled and 10% Na_2CO_3 (10 mL) was added to neutralize the *p*-TsOH salt and extracted with ethyl acetate (3×10 mL). The combined organic layer was given water wash (1×10 mL), dried over anhydrous Na_2SO_4, and evaporated under reduced pressure to obtain the free *N*-Me amino acid benzyl ester.

***N*-Me-Val-OBzl**: IR 1735 cm^{-1}; ^1H NMR (δ, $CDCl_3$) 0.91–0.95 (6H, m), 1.8 (1H, m), 1.5 (1H, br. s), 2.35 (3H, s), 3.4 (1H, m), 5.15 (2H, d), 7.2–7.4 (5H, m); ^{13}C NMR (δ $CDCl_3$) 18.7, 18.9, 29.2, 34.6, 42.2, 61.6, 66.2, 128.2, 128.5, 135.5, 175.8; ES MS m/z observed 236.3.

***N*-Me-Leu-OBzl**: IR 1738 cm^{-1}; ^1H NMR (δ, $CDCl_3$) 0.85–0.93 (6H, m), 1.45 (2H, m), 1.55 (1H, br. s), 1.65 (1H, m), 2.35 (3H, s), 3.25 (1H, t), 5.15 (2H, d), 7.28–7.38 (5H, m); ^{13}C NMR (δ $CDCl_3$) 22.3, 22.5, 24.8, 34.5, 42.5, 61.78, 66.19, 128.1, 128.4, 135.8, 175.2; ES MS m/z observed 250.4.

The spectral data of *N*-Me-D-Val-OBzl and *N*-Me-D-Leu-OBzl are satisfactory.

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