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Stability of Fmoc, Trt, and Boc protecting groups in charged droplets – electrospray ionization conditions

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ABSTRACT

We studied the stability of Fmoc, Trt, and Boc protecting groups under a variety of electrospray ionization (ESI) (+) or (–) conditions in ACN, MeOH, TFE, and DMF media or their mixtures with water in the presence of either acidic or basic additives, or without any additives. Generally, all the studied protecting groups remained stable under a majority of the studied conditions. We found that the cleavage of the Fmoc protecting group is possible under very mild conditions: aqueous DMF containing approximately 17% of water in the presence of TEA. The collected data will likely find usage in various ESI-MS and HPLC-MS applications, as well as in the chemistry of protecting groups and electrospray-mediated peptide synthesis.

ABBREVIATIONS

ACN – acetonitrile
Boc – tert-butyloxycarbonyl
DCM – dichloromethane
DMF – *N,N*-dimethylformamide
DMSO – dimethyl sulfoxide
ESI – electrospray ionization
EtOAc – ethyl acetate
Fmoc – 9-fluorenylmethyloxycarbonyl
Gln – glutamine
HPLC – high-performance liquid chromatography
MeOH – methanol
MS – mass spectrometry
PE – petroleum ether (light petroleum)
TEA – triethylamine
TFA – 2,2,2-trifluoroacetic acid
TFE – 2,2,2-trifluoroethanol
TLC – thin layer chromatography
Trt – triphenylmethyl
*p*TsOH – *p*-toluenesulfonic acid

Introduction

Charged droplets, generated during electrospray ionization (ESI), produce a unique and very promising reaction medium for various chemical processes. Indeed, the charged droplet approach often allows one to greatly enhance the reaction rate via concentration of the reaction medium, partial desolvation of reactants on the droplet surface [1–3,4], and generation of charge and superacidic or superbasic conditions leading to ionization of several very weakly basic or basic compounds [5]. Due to these effects, several successful applications of charged droplets in organic chemistry have been reported in literature. Among these are the Passerini reaction between isonitrile, aldehyde, and carboxylic acid being approximately 10^3 times faster than in bulk [6], the Pomeranz–Fritsch synthesis of isoquinoline resulting in the formation of the target heterocycle without acidic catalysis and being more than 10^6 times faster than the reaction in the bulk phase [7], and the Combes synthesis of quinolines being approximately 10^3 times faster [7].

The acidic catalyst-free version of the Pomeranz–Fritsch isoquinoline synthesis can be explained by the generation of strongly acidic media upon electrospraying [5], and it inspired us to test the stability of several acid- and base-labile protecting groups (Fmoc, Trt, and Boc), which are widely exploited in modern organic synthesis. Additionally, this study will broaden the possibilities for selective and mild

deprotection of several protected substrates sensitive under the common deprotection conditions, as well as allow one to estimate the stability of protected analytes during HPLC-MS and ESI-MS analyses, or even electrospray-mediated peptide synthesis.

Results and discussion

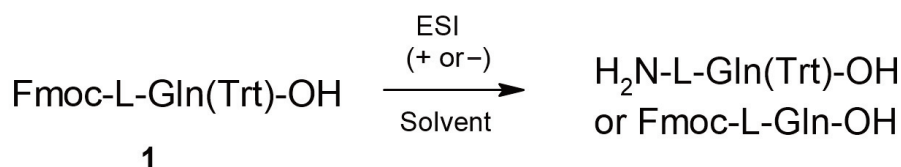
For our study, we took orthogonally protected Fmoc-L-Glutamine(Trt)-OH as a model substrate bearing the base-labile Fmoc group, usually removed by treatment with a primary or secondary amine [8], and the acid-sensitive Trt group, usually deprotected by 1%–5% TFA in DCM [9]. We used either neat ACN, water, MeOH, TFE, DMF, or mixtures of these polar solvents with water 5:1 (vol/vol) without or in the presence of TFA, HCOOH, *p*TsOH, or TEA. The principal idea is depicted in Scheme 1.

The starting electrospraying solution contained 30 mg of starting amino acid per 10 mL of solvent. For ESI experi-

ments, an in-house-built ESI setup was used. The ESI conditions utilized either a grounded collecting plate or a dipolar ESI, and the distance between the spraying needle and the collecting needle was 15 cm (Fig. 1).

The solutions were pumped using a syringe pump, the flow rates varied between 5–16 $\mu\text{L}/\text{min}$ (the majority of the experiments were performed using the flow rate of 16 $\mu\text{L}/\text{min}$; the flow rate was decreased when the electrospray cone was unstable), needle voltage was kept in the range of $+(-)8\text{--}17\text{ kV}$, and the collecting plate was either grounded or kept at voltage $+(-)5\text{ kV}$. All the parameters were adjusted in order to maintain a stable electrospraying cone. In an average experiment, approximately 150 μL of the initial amino acid solution was electrosprayed. The residue deposited on the collecting plate was dissolved in approximately 0.3 mL of commercial DMSO, and the obtained solution was analyzed by reverse-phase HPLC.

We started our work under ESI (+) protonating conditions in either the neat acetonitrile media or in the presence of water



Solvent: organic solvent (MeOH, TFE, ACN, DMF) or organic solvent + 17% H_2O or organic solvent + 17% H_2O + acidic (HCOOH or TFA or *p*TsOH) or basic (TEA) additive.
Concentration of additives: approx. 0.1%–3% by volume or mass (*p*TsOH).

Scheme 1. Aim of the study – possible mild deprotection of acid- and base-labile protecting groups under ESI (+) or (–) conditions.

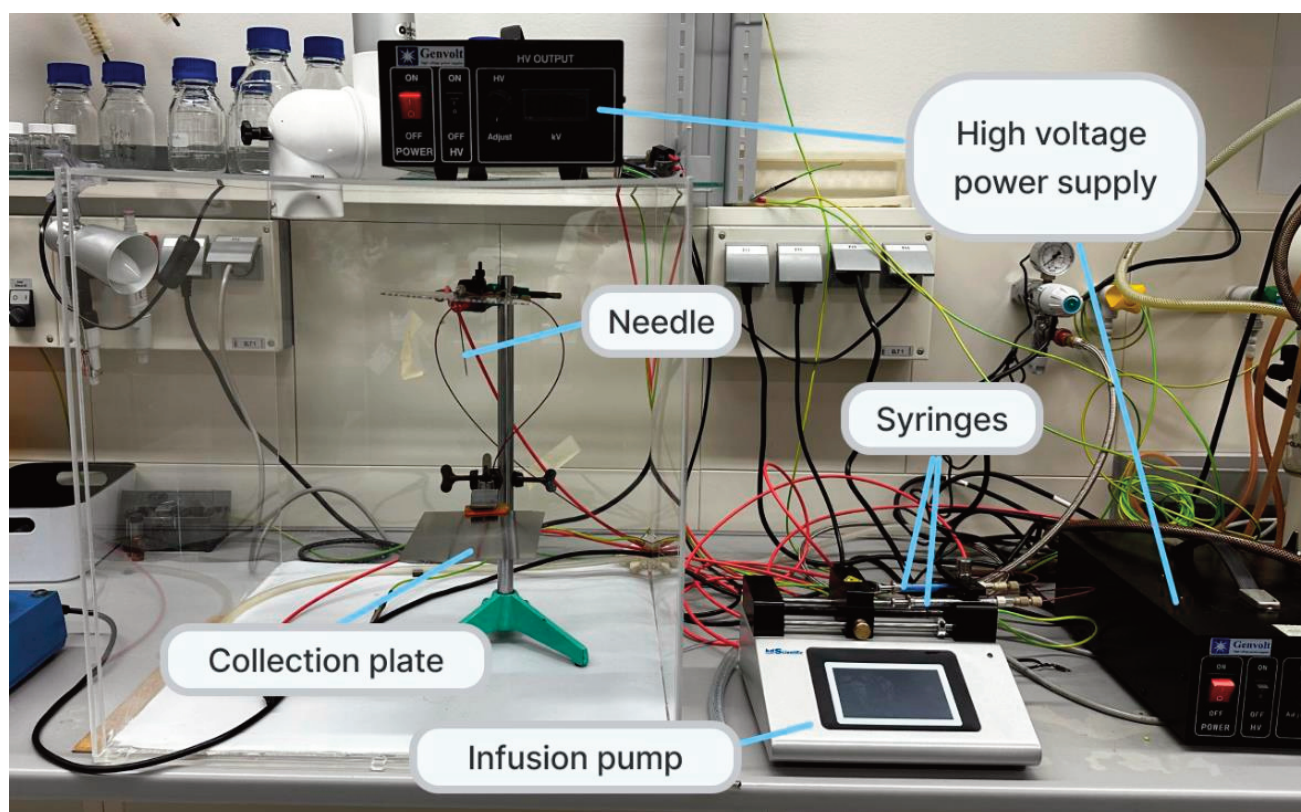


Fig. 1. In-house-built electrospray equipment.

Table 1. Stability of Fmoc-L-Gln(Trt)-OH under ESI (+) conditions in acetonitrile media

Entry	Needle voltage (kV)	Solvent	Collecting plate voltage (kV) or grounding conditions	Content of Fmoc-NH-Gln(Trt)-OH (HPLC, 254 nm)
A	HPLC analysis of starting material solution prior to electrospraying			97%
B	+12.1	ACN	Grounded	97%
C	+10.0	ACN	-5.0	98%
D	+12.1	ACN + 17% H ₂ O	Grounded	98%
E	+10.0	ACN + 17% H ₂ O	-5.0	98%
F	+12.4	ACN + 17% H ₂ O + 0.8% HCOOH	Grounded	98%
G	+10.0	ACN + 17% H ₂ O + 0.8% HCOOH	-5.0	98%
H	+14.2	ACN + (17% H ₂ O + 0.1% TFA)	Grounded	98%
I	+10.0	ACN + (17% H ₂ O + 0.1% TFA)	-5.0	97%
J	+12.7	ACN + 20% H ₂ O + 0.8% TFA	Grounded	98%
K	+12.7	ACN + 20% H ₂ O + 3% <i>p</i> TsOH (weight%)	Grounded	96%
L	Test HPLC analysis: HPLC analysis of the non-sprayed mixture after previous experiment			95%
M	+10.0	ACN + 20% H ₂ O + 3% <i>p</i> TsOH (weight%)	-5.0	96%

and several acidic additives. The results are summarized in Table 1.

Based on the obtained results (Table 1, entries B–K, M), the Trt protecting group is stable under a variety of ESI (+) conditions, and even strong non-volatile *p*TsOH (Table 1, entries K–M) did not result in the expected mild cleavage of the Trt protecting group. Test HPLC analysis (Table 1, entry L) revealed no remarkable deprotection of the Trt group in the syringe prior to electrospraying.

The following set of experiments was performed using ESI (–) deprotonating conditions in either the neat acetonitrile media or in the presence of water and a basic additive. The results are summarized in Table 2.

The obtained results (Table 2, entries B–E) demonstrated that the Fmoc group is stable under ESI (–) conditions in acetonitrile or aqueous acetonitrile media. Even the application of a small quantity of weakly nucleophilic TEA (Table 2, entries F and G) did not cause the cleavage of the Fmoc group. One of the possible explanations can be the neutralization of

TEA by the free carboxylic acid group. At the same time, the application of a larger quantity of TEA (Table 2, entries I and J) resulted in a slight cleavage of the Fmoc group, especially in the experiment utilizing the grounded collecting plate (Table 2, entry I). Test HPLC analysis of the non-sprayed mixture did not indicate remarkable removal of the Fmoc group in the presence of 0.8% TEA (Table 2, entry H); however, in the presence of a larger TEA quantity (Table 2, entry K), the content of Fmoc-L-Gln(Trt)-OH decreased by approximately 2% even without spraying. The latter observation can be explained by the losses of TEA during electrospraying and a longer reaction time.

The following experiments were performed under ESI (+) conditions with a grounded collecting plate and either neat TFE or its mixtures with water or water-containing acidic additives. The results are summarized in Table 3.

The collected results (Table 3, entries B–F) proved the stability of the Trt group under ESI (+) conditions in weakly acidic TFE (pK_a = 12.5 (in water)) [10]. Even the application

Table 2. Stability of Fmoc-L-Gln(Trt)-OH under ESI (–) conditions in acetonitrile media

Entry	Needle voltage (kV)	Solvent	Collecting plate voltage (kV) or grounding conditions	Content of Fmoc-NH-Gln(Trt)-OH (HPLC, 254 nm)
A	HPLC analysis of starting material solution prior to electrospraying			97%
B	-8.1	ACN	Grounded	97%
C	-8.1	ACN	+5.0	98%
D	-8.7	ACN + 17% H ₂ O	Grounded	98%
E	-8.1	ACN + 17% H ₂ O	+5.0	97%
F	-13.3	ACN + 17% H ₂ O + 0.8% TEA	Grounded	95%
G	-12.0	ACN + 17% H ₂ O + 0.8% TEA	+5.0	97%
H	Test HPLC analysis: HPLC analysis of the non-sprayed mixture after previous experiment			95%
I	-12.1	ACN + 17% H ₂ O + 3% TEA	Grounded	89%
J	-13.3	ACN + 17% H ₂ O + 3% TEA	+5.0	94%
K	Test HPLC analysis: HPLC analysis of the non-sprayed mixture after previous experiment			92%

Table 3. Stability of Fmoc-L-Gln(Trt)-OH under ESI (+) conditions in TFE media

Entry	Needle voltage (kV)	Solvent	Collecting plate voltage (kV) or grounding conditions	Content of Fmoc-NH-Gln(Trt)-OH (HPLC, 254 nm)
A	HPLC analysis of starting material solution prior to electrospraying			98%
B	+12.1	TFE	Grounded	98%
C	+10.0	TFE + 17% H ₂ O	Grounded	98%
D	+12.1	TFE + 17% H ₂ O + 0.8% HCOOH	Grounded	98%
E	+12.1	TFE + 17% H ₂ O + 0.8% TFA	Grounded	98%
F	+12.4	TFE + 17% H ₂ O + 3% TFA	Grounded	98%
G	+10.0	TFE + 17% H ₂ O + 2.5% <i>p</i> TsOH	Grounded	93%
H	Test HPLC analysis: HPLC analysis of the non-sprayed mixture after previous experiment			90%

of volatile HCOOH (Table 3, entry D) or TFA (Table 3, entries E and F) did not result in the cleavage of the Trt group. At the same time, the application of strong non-volatile *p*TsOH (Table 3, entry G) resulted in noticeable deprotection of the Trt group.

The control HPLC analysis of the non-sprayed mixture (Table 3, entry H) was performed approximately 1.3 h after the final ESI (+) experiment (Table 3, entry H) and revealed an even greater quantity of the Trt deprotected product. This result proved that the Trt group can be deprotected in a TFE-water mixture in the presence of a small quantity of *p*TsOH without ESI. The next set of experiments utilized TFE or TFE-rich media and the ESI (–) regime. The results are summarized in Table 4.

Based on the collected results (Table 4, entries B–I), the Fmoc group is stable in TFE media, and we were not able to detect Fmoc deprotection due to the concentration or formation of the basic medium. At the same time, test HPLC analysis of the TEA containing the reaction mixture (Table 4, entry J) performed after 1 h revealed a noticeable Fmoc cleavage.

Further, we focused on ESI (–) experiments in methanolic media using either neat methanol or its mixtures with water and some basic additives. The results are summarized in Table 5.

The collected data (Table 5, entries B–H, J) demonstrated that the Fmoc protecting group is stable under ESI (–) condi-

tions in methanolic media. HPLC analysis of the non-sprayed mixture (Table 5, entry I) indicated a moderate Fmoc cleavage.

The following experiments utilized ESI (+) conditions with a grounded collecting plate in methanolic media in the presence of acidic additives. The results are summarized in Table 6.

The collected data (Table 6, entries A–E) demonstrated the stability of the Trt protecting group under ESI (+) conditions in methanolic media and in the presence of water and acidic additives.

Next, we decided to explore the stability of the Fmoc and Trt groups under ESI (+) conditions in polar aprotic amide type solvents, such as DMF and its mixtures with water in the presence of acidic additives. The results are summarized in Table 7.

The collected results (Table 7, entries B–J) demonstrated the stability of the Trt protecting group under ESI (+) conditions in either neat DMF or its mixtures with water in the absence and in the presence of acidic additives. HPLC analysis of the non-sprayed mixture in the presence of volatile TFA (Table 7, entry K) did not show noticeable deprotection of the Trt group. At the same time, both experiments in the presence of *p*TsOH (Table 7, entries L and M) resulted in a slightly decreased content of Fmoc-L-Gln(Trt)-OH. Test HPLC analysis of the non-sprayed *p*TsOH-containing mixture after the

Table 4. Stability of Fmoc-L-Gln(Trt)-OH under ESI (–) conditions in TFE media

Entry	Needle voltage (kV)	Solvent	Collecting plate voltage (kV) or grounding conditions	Content of Fmoc-NH-Gln(Trt)-OH (HPLC, 254 nm)
A	HPLC analysis of starting material solution prior to electrospraying			98%
B	–8.1	TFE	Grounded	98%
C	–8.1	TFE	+5.0	97%
D	–8.1	TFE + 17% H ₂ O	Grounded	98%
E	–8.2	TFE + 17% H ₂ O	+5.0	98%
F	–11.5	TFE + 17% H ₂ O + 0.8% TEA	Grounded	97%
G	–8.1	TFE + 20% H ₂ O + 0.8% TEA	+5.0	98%
H	–12.2	TFE + 17% H ₂ O + 3% TEA	Grounded	96%
I	–8.1	TFE + 17% H ₂ O + 3% TEA	+5.0	95%
J	Test HPLC analysis: HPLC analysis of the non-sprayed mixture after previous experiment			88%

Table 5. Stability of Fmoc-L-Gln(Trt)-OH under ESI (–) conditions in methanolic media

Entry	Needle voltage (kV)	Solvent	Collecting plate voltage (kV) or grounding conditions	Content of Fmoc-NH-Gln(Trt)-OH (HPLC, 254 nm)
A	HPLC analysis of starting material solution prior to electrospraying			98%
B	–9.2	MeOH	Grounded	98%
C	–8.1	MeOH	+5.0	98%
D	–8.1	MeOH + 17% H ₂ O	+5.0	98%
E	–10.0	MeOH + 17% H ₂ O	Grounded	98%
F	–13.6	MeOH + 17% H ₂ O + 0.8% TEA	Grounded	97%
G	–11.8	MeOH + 17% H ₂ O + 0.8% TEA	+5.0	97%
H	–11.8	MeOH + 17% H ₂ O + 3% TEA	Grounded	97%
I	Test HPLC analysis: HPLC analysis of the non-sprayed mixture after previous experiment			93%
J	–11.8	MeOH + 17% H ₂ O + 3% TEA	+ 5.0	97%

Table 6. Stability of Fmoc-L-Gln(Trt)-OH under ESI (+) conditions in methanolic media

Entry	Needle voltage (kV)	Solvent	Collecting plate voltage (kV) or grounding conditions	Content of Fmoc-NH-Gln(Trt)-OH (HPLC, 254 nm)
A	+13.0	MeOH	Grounded	98%
B	+13.5	MeOH + 17% H ₂ O	Grounded	99%
C	+14.4	MeOH + 5% HCOOH	Grounded	97%
D	+13.4	MeOH + 5% TFA	Grounded	98%
E	+14.7	MeOH + 20% H ₂ O + 5% TFA	Grounded	98%

Table 7. Stability of Fmoc-L-Gln(Trt)-OH under ESI (+) conditions in DMF media

Entry	Needle voltage (kV)	Solvent	Collecting plate voltage (kV) or grounding conditions	Content of Fmoc-NH-Gln(Trt)-OH (HPLC, 254 nm)
A	HPLC analysis of starting material solution prior to electrospraying			97%
B	+12.2	DMF	Grounded	97%
C	+8.1	DMF	–5.0	97%
D	+10.0	DMF + 17% H ₂ O	–5.0	97%
E	+12.8	DMF + 17% H ₂ O + 0.8% HCOOH	Grounded	97%
F	+8.6	DMF + 20% H ₂ O + 0.8% HCOOH	–5.0	97%
G	+8.6	DMF + 17% H ₂ O + 0.8% TFA	–5.0	97%
H	+12.0	DMF + 17% H ₂ O + 0.8% TFA	Grounded	97%
I	+13.0	DMF + 17% H ₂ O + 3% TFA	Grounded	97%
J	+8.6	DMF + 20% H ₂ O + 3% TFA	–5.0	97%
K	Test HPLC analysis: HPLC analysis of the non-sprayed mixture after previous experiment			96%
L	+12.7	DMF + 20% H ₂ O + 3% <i>p</i> TsOH	–5.0	95%
M	+13.4	DMF + 20% H ₂ O + 3% <i>p</i> TsOH	Grounded	94%
N	Test HPLC analysis: HPLC analysis of the non-sprayed mixture after previous experiment			91%

ESI (+) experiment (Table 7, entry N) demonstrated an even lower content of the starting orthogonally protected amino acid, meaning that slow Trt group deprotection happens without ESI conditions.

Subsequently, we tested the Fmoc group under ESI (–) conditions in either DMF or DMF-water in the presence of

or without a TEA additive. The results are summarized in Table 8.

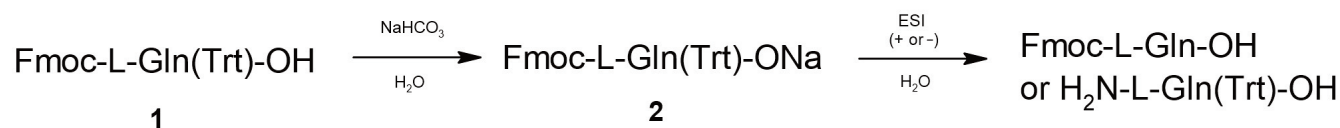
The collected data demonstrated the stability of the Fmoc groups under ESI (–) conditions in neat or aqueous DMF (Table 8, entries B–E). The addition of a smaller quantity of TEA resulted in a slight decrease in the Fmoc-L-Gln(Trt)-OH

Table 8. Stability of Fmoc-L-Gln(Trt)-OH under ESI (–) conditions in DMF media

Entry	Needle voltage (kV)	Solvent	Collecting plate voltage (kV) or grounding conditions	Content of Fmoc-NH-Gln(Trt)-OH (HPLC, 254 nm)
A	HPLC analysis of starting material solution prior to electrospraying			97%
B	–10.1	DMF	Grounded	96%
C	–10.0	DMF	+5.0	96%
D	–10.0	DMF + 17% H ₂ O	+5.0	96%
E	–12.8	DMF + 17% H ₂ O	Grounded	96%
F	–16.9	DMF + 17% H ₂ O + 0.8% TEA	Grounded	92%
G	–10.0	DMF + 17% H ₂ O + 0.8% TEA	+5.0	92%
H	–17.0	DMF + 17% H ₂ O + 3% TEA	+5.0	91%
I	–17.0	DMF + 17% H ₂ O + 3% TEA	Grounded	89%
J	Test HPLC analysis: HPLC analysis of the non-sprayed mixture after previous experiment			70%

content in the electrosprayed product independently of the collecting plate conditions (Table 8, entries F and G). Further increase in the TEA content (Table 8, entries H and I) resulted in a more extensive Fmoc cleavage, and the experiment utilizing the grounded collecting plate (Table 8, entry I) gave more Fmoc-deprotected product. Surprisingly, the test HPLC analysis of the non-sprayed TEA-containing mixture revealed excessive Fmoc removal taking place in the aqueous DMF even without spraying (Table 8, entry J). The latter conditions can be utilized for a very mild cleavage of the Fmoc group and, after additional optimization, could find application if the protected substrate is sensitive to standard Fmoc deprotection conditions.

Based on the previous results (Tables 1–8), we thought that one of the possible reasons for the mostly negative ESI results might be the difficult protonation of the orthogonally protected amino acid. In order to prove this idea, we prepared water-soluble Fmoc-L-Gln(Trt)-ONa (Scheme 2: 2) by treatment of an aqueous suspension Fmoc-L-Gln(Trt)-OH (Scheme 2: 1) with a small quantity of a saturated NaHCO₃ solution and tested the obtained opaque and foaming solution under ESI (+) and (–) conditions (Scheme 2). The results are summarized in Table 9.

**Scheme 2.** Preparation and ESI (+) or (–) stability of Fmoc-L-Gln(Trt)-ONa in aqueous medium.**Table 9.** Stability of Fmoc-L-Gln(Trt)-ONa under ESI (+) or (–) conditions in aqueous medium

Entry	Needle voltage (kV)	Solvent	Collecting plate voltage (kV) or grounding conditions	Content of Fmoc-NH-Gln(Trt)-OH (HPLC, 254 nm)
A	HPLC analysis of starting material solution prior to electrospraying			97%
B	–14.8	H ₂ O	Grounded	97%
C	–14.8	H ₂ O	+5.0	97%
D	+14.0	H ₂ O	Grounded	97%
E	+14.0	H ₂ O	–5.0	96%

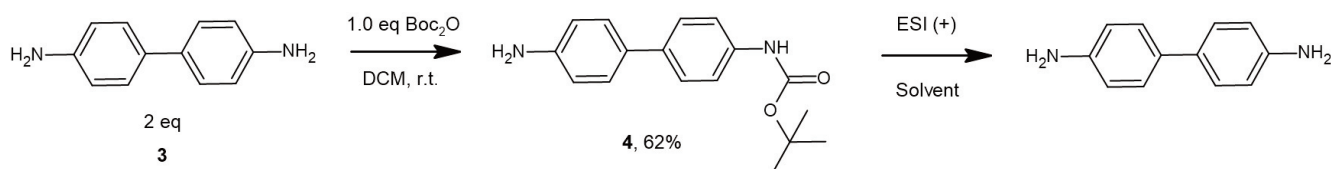
The collected data (Table 9, entries B–E) demonstrated great stability of both protecting groups in aqueous medium independently of ESI polarity. Finally, we decided to test the stability of the Boc protecting group under ESI (+) conditions in methanolic media. For this purpose, we synthesized mono-Boc-protected benzidine (Scheme 3: 4) by treatment of excess benzidine (Scheme 3: 3) with 1 eq of Boc₂O in DCM medium. The target mono-Boc-protected product (Scheme 3: 4) was obtained in 62% yield. The results are summarized in Table 10.

The collected data (Table 10, entries B–F) proved the stability of the NH-Boc protecting group under ESI (+) conditions in either neat methanol or its mixtures with water without or in the presence of acidic additives.

Experimental

General

All solvents and reagents were purchased from Merck, Sigma-Aldrich, or Lach-Ner (HPLC or synthesis grade) and used without additional purification or drying. Water content was not additionally quantified. Melting points were obtained on the Stuart SMP10 capillary apparatus and are uncorrected. Nuclear magnetic resonance (NMR) spectra were measured on



Solvent: MeOH, MeOH + 17% H₂O or MeOH + 17% H₂O + acidic additives.

Scheme 3. Preparation of mono-Boc-protected benzidine and its stability under ESI (+) conditions.

Table 10. Stability of mono-Boc-protected benzidine under ESI (+) conditions in methanolic media

Entry	Needle voltage (kV)	Solvent	Collecting plate voltage (kV) or grounding conditions	Content of mono-Boc benzidine (HPLC, 254 nm)
A	HPLC analysis of starting material solution prior to electrospraying			98%
B	+8.5	MeOH	Grounded	98%
C	+11.8	MeOH + 17% H ₂ O	Grounded	98%
D	+12.2	MeOH + 17% H ₂ O + 0.1% TFA	Grounded	97%
E	+13.1	MeOH + 17% H ₂ O + 3% HCOOH	Grounded	98%
F	+12.7	MeOH + 17% H ₂ O + 3% TFA	Grounded	98%

a Bruker spectrometer (700 MHz for ¹H spectra and 176 MHz for ¹³C spectra), using DMSO-d₆ as a solvent and solvent residual signal as the internal reference. IR spectra were recorded using attenuated total reflectance (ATR) measuring technique on a PerkinElmer Spectrum BX spectrometer.

The reaction progress was monitored using thin layer chromatography (TLC) on silica gel 60 F₂₅₄ TLC plates (Merck). TLC plates were visualized under UV light (254 nm) and by basic KMnO₄ staining solution. Column chromatography was performed on Silica Gel 60 Å (63–200 μm, Supelco). All of the yields reported were based on the masses of the starting reagents. HPLC analyses were performed on a Shimadzu Prominence System equipped with a Phenomenex 2.6 μm column (C18, 100 Å, 150 × 4.6 mm) using the following elution method at ambient temperature: A – water + 0.1% TFA; B – ACN; 0–5 min, 5% B; 5–32 min, 5%–100% B; detection at 254 nm. HPLC was not calibrated, the content of Fmoc-NH-Gln(Trt)-OH was determined by simple integration of peaks, and reproducibility was not studied. The precision of all the HPLC analyses corresponds to a semi-quantitative analysis.

General procedure for ESI experiments

The starting electrospraying solution contained 30 mg of starting amino acid per 10 mL of solvent. If either basic or acidic additives were utilized, their concentration was in the range 0.1%–3% (volume% for liquid additives and weight% for solid additives). For ESI experiments, an in-house-built ESI setup was used. The ESI conditions utilized either a grounded collecting plate or a dipolar ESI, and the distance between the spraying needle and the collecting plate was 15 cm. The solutions were pumped using a syringe pump, flow rates varied between 5–16 μL/min (the majority of the experiments were performed using the flow rate of 16 μL/min; the flow rate was decreased when the electrospray cone was unstable), needle voltage was kept in the range +(–)17–8 kV in order to maintain a stable electrospraying, and the collecting plate was

either grounded or kept at voltage +(–)5 kV. All the parameters were adjusted in order to maintain a stable electrospraying cone. In an average experiment, approximately 150 μL of the initial amino acid solution was electrosprayed. The residue deposited on the collecting plate was dissolved in approximately 0.3 mL of commercial DMSO, and the obtained solution was analyzed by reverse-phase HPLC, using the elution conditions described above.

Synthesis of tert-butyl (4'-amino[1,1'-biphenyl]-4-yl)carbamate (mono-Boc benzidine) (4)

Benzidine (2 eq, 0.30 g, 1.63 mmol) was weighed into a 10 mL round-bottom flask equipped with a stirrer bar and suspended in 4 mL of commercial DCM (some benzidine remained undissolved). The reaction flask was flushed with N₂ and isolated from atmosphere by a N₂-filled balloon. 1 eq of Boc₂O (173 μL) was dissolved in 2 mL of commercial DCM, and the obtained solution was added dropwise to the stirring suspension of benzidine. After the addition was complete, the obtained reaction mixture was left to stir overnight at room temperature. The next day, the reaction progress was checked by TLC using EtOAc/PE 1:1 as the eluent. The eluted chromatogram indicated the formation of the target mono-Boc-protected product and excess of the starting material (R_f (product) = 0.48; R_f (benzidine) = 0.26). Next, the crude reaction mixture was rotary-evaporated to approximately 50% of its initial volume at 45 °C, and the obtained concentrate was purified by column chromatography on silica using EtOAc/PE 1:1 as the eluent. Evaporation of the target fractions followed by vacuumation in a lyophilizer afforded 144 mg (62%) of the desired tert-butyl (4'-amino[1,1'-biphenyl]-4-yl)carbamate as a yellow solid.

Conclusion

To sum up, we studied the stability of Fmoc, Trt, and Boc protecting groups under a variety of ESI (+) or (–) conditions

in ACN, MeOH, TFE, and DMF media or their mixtures with water in the presence of either acidic or basic additives or without any additives. Generally, all the studied protecting groups remained stable under the majority of studied conditions. It was found that the cleavage of the Fmoc protecting group is possible under very mild conditions: aqueous DMF containing approximately 17% of water in the presence of TEA. The collected data will likely find usage in various ESI-MS and HPLC-MS applications as well as in the chemistry of protecting groups and electrospray-mediated peptide synthesis.

Notes

mp 149–150 °C, lit. [11] 145 °C, R_f (EtOAc/PE 1:1) = 0.48. IR (cm^{-1}): 3365, 3310, 3211, 3039, 2988, 1698, 1613, 1503, 1269, 1229, 1155, 1057, 813, 767, 755, 656. NMR (700 MHz, DMSO- d_6): ^1H (700 MHz) δ = 1.48 (s, 9H, $3 \times \text{CH}_3$), 5.13 (s, 2H, NH_2), 6.62 (dt, 2H, $J_1 = 8.4$ Hz, $J_2 = 2.1$ Hz, Ar(H)), 7.30 (dd, 2H, $J_1 = 7$ Hz, $J_2 = 2.1$ Hz), 7.42 (d, 2H, $J = 8.4$ Hz, Ar(H)), 7.45–7.46 (m, 2H, Ar(H)), 9.30 (s, 1H, NH); ^{13}C (176 MHz) δ = 28.1, 78.9, 114.2, 118.5, 125.4, 126.6, 127.3, 134.6, 137.4, 147.8, 152.8.

Data availability statement

All data are contained in the article.

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Fmoc, Trt ja Boc kaitserühma stabiilsus laetud tilkades elektropihustusionisatsiooni tingimustes

Anton Mastitski ja Peeter Burk

Uurimustöö raames tehti põhjalik Fmoc, Trt ja Boc kaitserühmade stabiilsuse uuring erinevates elektropihustusionisatsiooni (ESI) (+)- või (–)-tingimustes. Lahustitena kasutati ACN-i, MeOH-d, TFE-d ja DMF-i või nende segusid veega kas happeliste või aluseliste lisandite juuresolekul või ilma lisanditeta. Üldiselt talusid kõik uuritud kaitserühmad suuremat osa kasutatud tingimustest. Leiti, et Fmoc kaitserühma eemaldamine on võimalik väga pehmetes tingimustes: vesises DMF-is TEA juuresolekul. On tõenäoline, et saadud tulemused leiavad kasutust erinevates elektropihustus-massispektromeetria (ESI-MS) ja kõrgefektiivse vedelikromatograafia-massispektromeetria (HPLC-MS) rakendustes, kaitserühmade keemias ning elektropihustust kasutavas peptiidsünteesis.
