TFA-Free Cleavage and Deprotection in Solid-Phase Peptide Synthesis: Advantages and Drawbacks of Formic Acid

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Introduction

Solid-phase synthesis is one of the main techniques for the production of peptides applied in the pharmaceutical and cosmetic industries. However, the synthetic process relies on the application of trifluoroacetic acid (TFA) at the stage of global peptide deprotection and cleavage from the polymeric support. Beyond its health and environmental hazards, TFA increases operational costs by requiring strict safety measures, waste disposal, and counterion conversion into pharmaceutically acceptable acetate or hydrochloride forms.

Due to growing evidence of accumulation in surface waters and resistance to biodegradation, TFA use faces imminent regulatory restrictions by the European Chemicals Agency. Nevertheless, its application remains unavoidable for industrial-scale peptide production via SPPS.

Herein, we present a PFAS-free cleavage and deprotection protocol* based on *in situ* generation of HCl in formic acid, yielding the final product in a pharmaceutically acceptable salt form.

* Patent-pending technology

Results and Discussion

Analysis of the literature suggests the utility of formic acid (FA) as the primary component of deprotection systems. Owing to its acidity (pK_a 3.75), it can be applied in peptide chemistry for the removal of Boc and *t*Bu protecting groups[1,2].

Our preliminary experiments demonstrated that neat FA enables the removal of Trt protecting groups from Gln, Asn, and His (**Table 1**). However, achieving complete deprotection requires heating the reaction mixture to 40-50 ^{0}C, depending on the peptide structure.

These results prompted us to reinvestigate the utility of HCl/FA for peptide deprotection and cleavage from the solid support. Therefore, subsequent experiments were performed on the most challenging models: Pbf removal from Arg and the cleavage of Fmoc-Phe-OH from Wang resin.

It was shown that deprotection efficiency strongly depends on the water content in the reaction mixture (**Table 2**). The best results were obtained by generating HCl *in situ* through mixing of anhydrous FA with TMSCl or related reagents (**Tables 1 and 3**).

The practical utility of this approach was tested on a number of peptides for both pharmaceutical and cosmetic applications. It was shown that using of the TMSCl/FA mixture significantly reduced Met alkylation compared to the standard TFA treatment (**Fig. 1**).

While peptide deprotection is accompanied by partial Ser, Thr, Tyr, and Trp formylation, the desired product is easily recovered by treatment with nucleophilic agents (**Fig. 2 and 3A**). Lys residue formylation via formyl group transfer from the adjacent hydroxy acid can be suppressed using an appropriate nucleophile (**Fig. 3B and Table 4**).

Deprotection of some Trp-containing peptides was accompanied by an unexpected side reaction: C-2 alkylation of Trp with the bulky Trt cation. However, the side-product formation can be efficiently suppressed by adding TIS to the reaction mixture (**Fig. 5**).

It was shown that the purity and yield of the model peptides after TMSCl/FA deprotection were at least comparable to those obtained via the standard TFA procedure **(Table 5**).

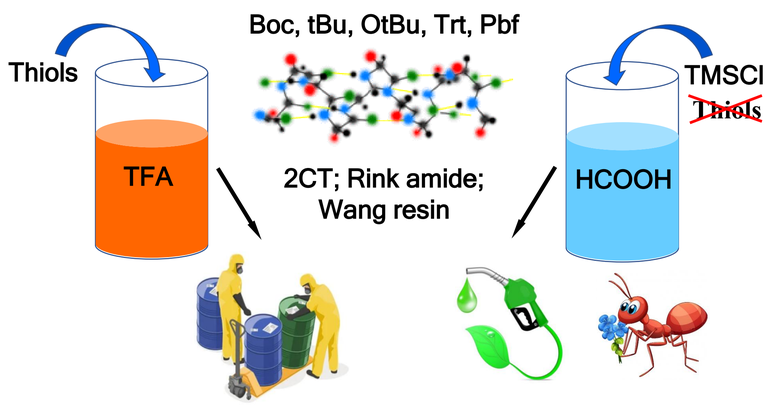


Table 2. Influence of water content on Arg(Pbf) deprotection

Deprotection conditions	HCOOH (%)	Fmoc-Arg(Pbf)-OH (%)		
		30 min	1 h	2 h
1 M HCl(aq)/FA	85	98	94	88
1 M HCl(aq)/FA	90	92	81	69
1 M HCl(aq)/FA	98	45	6	0.4
1 M TMSCl/FA	98	7	0.6	0.1

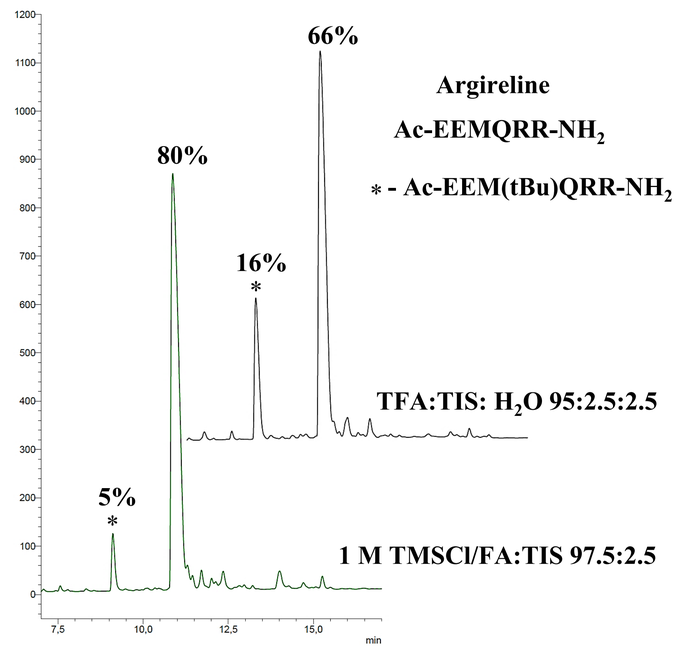


Fig. 1. Peptide deprotection in FA reduces Met alkylation.

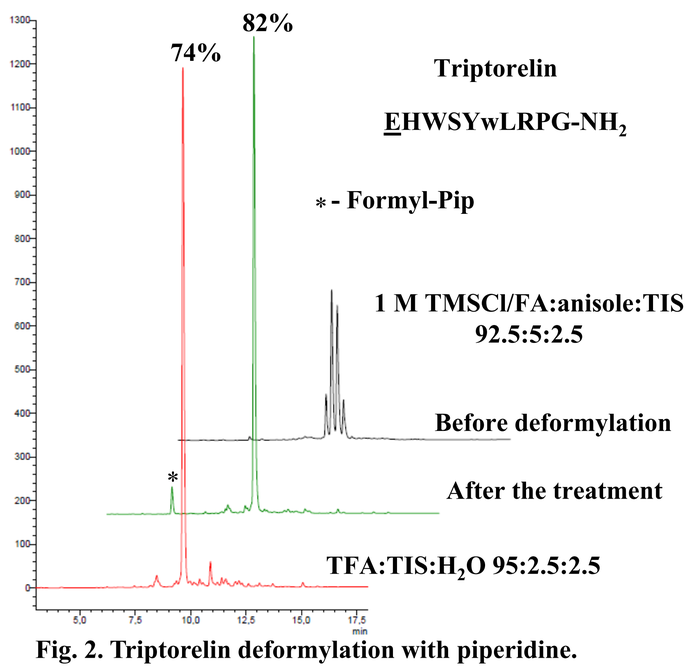


Fig. 2. Triptorelin deformylation with piperidine.

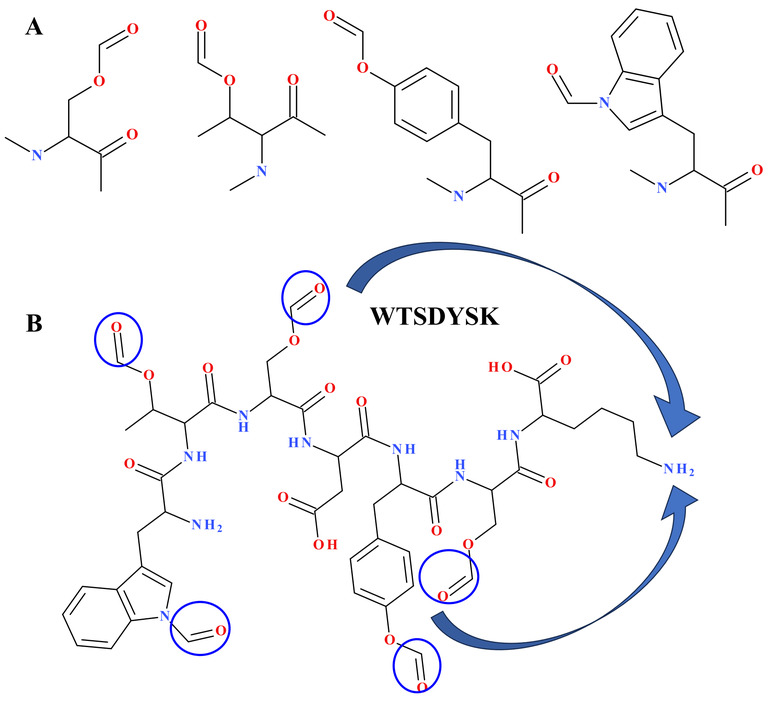


Fig. 3. Formation of primary (A) and secondary (B) side products.

Table 4. Recovery of the final product

Reagent	Lys formylation (%)
Piperidine	**18.6**
Piperazine	**8.5**
Morpholine	**3.7**
Substance "H"	**0.3**

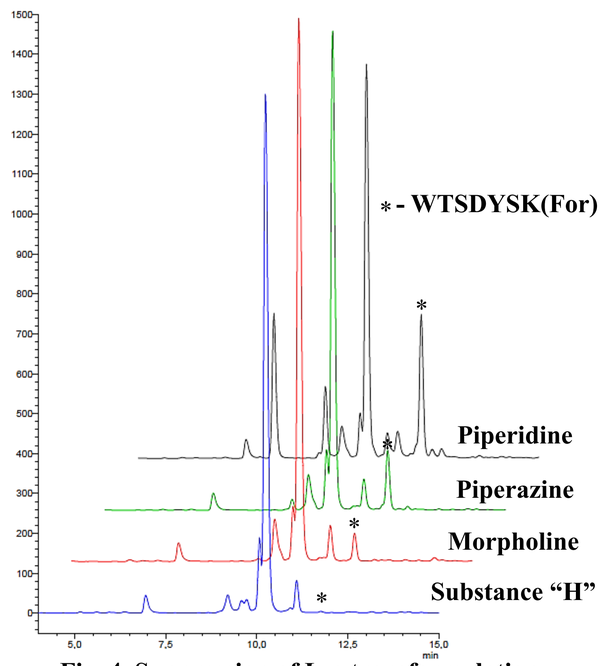


Fig. 4. Suppression of Lys transformylation.

Table 1. Removal of side-chain protecting groups

Protecting group	Amino acids	Deprotection conditions	
		FA	TMSCl/FA
^{t}Bu	Ser, Thr	30 min, RT	<30 min, RT
	Tyr	30 min, RT	30 min, RT
O^{t}Bu	Glu, Asp	30 min, RT	30 min, RT
BOC	Lys, Orn	1 h, RT	30 min, RT
	Trp	30 min, RT	30 min, RT
Trt	His	3 h, 50 ^{0}C	3 h, 40 ^{0}C
	Gln, Asn	2 h, 50 ^{0}C	1 h, RT
	Cys	Stable	2 h, RT (5% TIS)
Pbf	Arg	Stable	2 h, RT

Table 3. Cleavage yields (%) from Fmoc-Phe-resin after 2 h

Cleavage cocktail	Rink amide AM resin	Wang resin	
	RT	RT	40 ^{0}C
95% TFA/H_2O	78	41	92
1 M HCl(aq)/FA	63	15	89
1 M TMSCl/FA	84	79	93
1 M TMSBr/FA	93	96	95

TMSCl/FA vs. TFA deprotection

Entry	TFA	TMSCl/FA
Cleavage efficiency	✓ High	✓ High
Aspartimide formation	✓Low	Moderate
Reagents cost	High	✓ Moderate
Waste management	Costly	✓Cheap
Environmental hazard	High	✓ Moderate
Toxicity	High	✓Moderate
Counterion	TFA	✓HCl

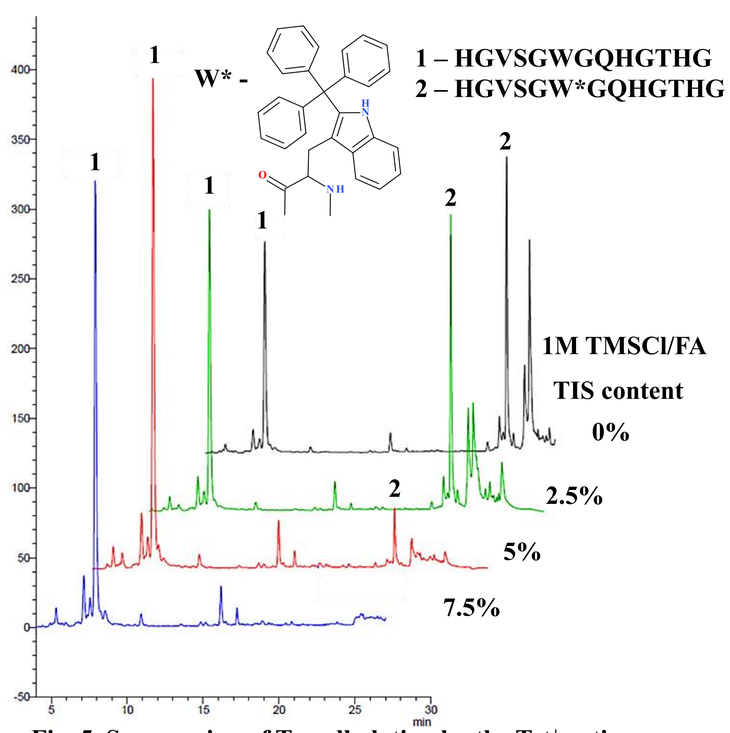


Fig. 5. Suppression of Trp alkylation by the Trt$^+$ cation.

Table 5. Deprotection of the model peptides

Peptide sequence	Brand name	Resin	Purity (%)[a]	
			A	B
Pal-HfR-NH_2	Neutrazen	Rink amide AM	91	91
Pal-HfRW-NH_2	Greyverse	Rink amide AM	77	84
GLYYF-NH_2	P5N	Rink amide AM	90	89
Ac-EEMQRR-NH_2	Argireline	Rink amide AM	86	89
deamino-CYFQNCPrG-NH_2[b]	Desmopressin	Rink amide AM	40	55
EHWSYwLRPG-NH_2	Triptorelin	Rink amide AM	74	82
HGVSGWGQHGTHG	Allostatin	2CT	69	76
		Wang	59	71
PKKKRKV$_\beta$AHAIYPRH-NH_2	–	Rink amide AM	84	86
YLQDQARLNSWGCAFRQVC-NH_2[c]	HIV-2-19	Rink amide AM	34	34

[a] Purity of the crude product after deformylation. [b] Linear precursor.
[c] Acm protected (synthesis was not optimized). A – TFA cleavage protocol; B – 1 M TMSCl/FA.

Conclusions

- **The proposed PFAS-free procedure, based on *in situ* generation of HCl in formic acid, enables global peptide deprotection and cleavage from Rink amide and Wang resins.**
- **This synthetic protocol utilizes inexpensive, readily available reagents and simplifies hazardous waste disposal.**

References

1. Kinoshita, H.; Kotake H. *Chemistry Letters*., **1974**, *3*, 631–634.
2. Toonstra, C.; Amin, M. N.; Wang L.-X. *J Org Chem*. **2016**, *81*, 6176–6185.