## *Confirmation that there is no current alternative to TFA for peptide synthetic process*

Trifluoroacetic acid (TFA) is an essential reagent for the peptide synthesis process, either in liquid or solid-phase fashion. The advent of the so-called Fmoc/tBu protection scheme in the 1970s introduced this organic acid as a safer reagent to assist side-chain and C-terminal protection removal (either from a solid-support or in Ajinamoto liquid-phase strategy) in substitution of hydrogen fluoride (HF), an extremely corrosive, skin-penetrating and blindness-causing gas, which requires specific training for manipulation.[1,2,3,4] Thus, the milder, less toxic and non-bioaccumulative TFA played a major role in adapting peptide synthesis to manufacture scales, therefore allowing the widespread dissemination of peptides as active ingredients in the chemical industry, including cosmetic business. TFA also offers advantages in terms of Green Chemistry: allows for cleavage and deprotection reactions to proceed at room temperature, saving thermal energy and avoiding potential runaways. From the safety standpoint, as a liquid it can be easily manipulated and does not hold the potential to trigger fire in contrast to several inorganic acids with are often strong oxidizers.

To date, TFA still plays an indispensable part in the peptide process in spite of the research conducted in the preceding decades seeking to reduce environmental impact of the synthesis. Not only is essential for cleavage of the peptide from the solid support but also paramount in liberating the full, native unprotected sequence required for biological applications.[5] In fact, the current lack of suitable alternatives to TFA for acid removal of permanent protecting groups is the main hurdle that hampers industry-scale implementation of TFA-free SPPS protocols. While some TFA-free conditions have been reported to perform cleavage and side-chain removal, the inclusion of PFAS such as fluoroalcohols (TFE/HFIP) or other fluorinated acids (TFMSA) is mandatory.[6,7,8] Such choice of reagents is far from trivial: the electronic interactions offered by fluorine are unparalleled in non-PFAS organic compounds. Fluorine behaves as an excellent hydrogen bond acceptor, surpassing the ability of other halogens or even oxygen and nitrogen, and is also unique at peptide solvation.[9,10,11] Consequently, fluoroalcohols can interact with acids in the cleavage cocktail through hydrogen bonding, enhancing its potency.[12] Reaction conditions without fluoroalcohols dramatically impact efficiency and even using these fluorinated compounds, evolution to the target unprotected peptide doesn't reach completion, therefore not matching the performance of TFA.[8] Consequently, the question on how to perform TFA- and PFA-free cleavage plus side-chain removal using suitable commercial building blocks remains unsolved and extremely challenging, as has been acknowledged recently by world-renowned experts in the field.[5]

Taking into account the PFAS-limited range of acidic conditions for full cleavage and protecting group removal, extensive research has been conducted on various orthogonal protection modalities for each amino acid.[13] However, building blocks displaying acid-sensitive side-chain group (i.e. removed by TFA) are still the only ones offering availability, efficiency and compatibility with production and business requirements. In example, arginine protection as Fmoc-Arg(Alloc)$_2$-OH and Fmoc-Arg($NO_2$)-OH avoid the necessity for TFA but are either discontinued in most commercial sources, impractical in terms of cost or

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[1] Chang, C.-D.; Meienhofer, J. *Int. J. Peptide Protein Res* **1978**, *11*, 246

[2] Atherton, E.; Fox, H.; Harkiss, D.; Sheppard,R.C. *J. Chem.Soc. Chem. Commun*. **1978**, 537

[3] https://emergency.cdc.gov/agent/hydrofluoricacid/basics/facts.asp

[4] Muttenthaler, M.; Albericio, F.; Dawson, P. E. *Nat.Protoc*. **2015**,10, 1067

[5] Al Musaimi, O.; García de la Torre, B.; Albericio, F. *Green Chem*., **2020**,22, 996

[6] Barlos, K.; Chatzi, O.; Gatos, D.; Stavropoulos, G. *Int. J. Peptide Protein Res*. 37, **1991**, 513-520

[7] Bollhagen, R.; Schmiedberger, M.; Barlos, K.; Grell. E. *J. Chem. Soc., Chem. Commun.,* **1994**, 2559

[8] Palladino, P.; Stetsenko, D.A. *Org. Lett*. **2012**, 14, 6346.

[9] Ballinger, P.; Long, F. A. *J. Am. Chem. Soc.* **1959**, 81, 1050.

[10] Kovács, A.; Varga, Z. *Coordination Chemistry Reviews*, **2006**, 250, 710.

[11] Chatterjee, C.; Gerig, J. T. *Biopolymers* **2007**, 87, 115.

[12] Prakash, G. K. S.; Mathew, T.; Marinez, E. R.; Esteves, P. M.; Rasul, G.; Olah, G. A. *J. Org. Chem.* **2006,** 71, 3952.

[13] Isidro-Llobet, A.; Álvarez, M.; Albericio, F. *Chem. Rev.* **2009**, 109 (6), 2455.

require dangerous, difficult to scale-up chemistries.[13] Not to mention that not all proteinogenic acids have an alloc- or $H_2$-friendly available version (such as the case of Gln).

It is our vision to seek for greener, more sustainable and environmentally friendly alternatives to TFA that avoid polyfluoroalkyl substances. However, in light of the abovementioned handicaps, such thorough and critical process modification cannot take place in a few years on an industrial level but rather requires a longer time. Substitution of the current building blocks, reagents and resins for TFA-free friendly counterparts demands extensive screening and finding new chemistries that also work on a multi-Kg scale and that are competitive in terms of cost. Therefore, a derogation period of the maximum possible duration is necessary to implement non-TFA peptide synthesis without compromising cosmetic business.