opn2EXPERTS - Eco-friendly TFA-free solid-phase peptide synthesis

How would you propose to avoid the use of trifluoroacetic acid (TFA) for resin cleavage and deprotection of synthetic peptides using an alternative reagent cocktail, to advance more sustainable peptide synthesis?

Answers to this question including a proposal for collaboration can only be considered if they arrive no later than May 28, 2024, 11:59 pm PST.

What is the context of the problem that we would like to solve?

In recent years, newly approved peptide therapeutics have revolutionized treatments across various therapeutic areas. Most notably, a new generation of long-acting incretins has emerged, offering unprecedented efficacy in glycemic control and body weight management. These new products have quickly risen to become some of the best-selling drugs on the market.

However, the success of these new peptide therapeutics has introduced new challenges for supply chains. Peptide manufacturers are now tasked with producing high-quality peptide APIs in large quantities. Furthermore, in an era increasingly defined by sustainability, these manufacturers are expected to adopt practices that promote the efficient use of raw materials, reduce energy consumption, and minimize waste streams.

Conventional peptide synthesis makes use of an insoluble carrier polymer to facilitate the consecutive coupling of amino acid building blocks according to the desired peptide sequence (known as solid phase peptide synthesis, SPPS). After assembly of the peptide chain, concentrated **trifluoroacetic acid** (TFA) is used as the standard reagent for detaching the crude peptide from the solid support and simultaneously removing all semi-permanent

side chain protecting groups in a single step. This takes advantage of the fact that solid-phase resins show good swelling behavior and peptides are well soluble in TFA.

Scavenger agents are routinely added to mitigate side-reactions triggered by reactive intermediates formed during deprotection.

However, TFA is non-biodegradable and behaves as a very persistent and very mobile (vPvM) substance when introduced into the environment. TFA concentrations in surface water samples have risen significantly in recent decades[1,2]. In light of mounting evidence of environmental accumulation of per- and polyfluorinated alkyl substances (PFAS), a legislative initiative has been launched to prohibit the production and use of this class of compounds in the EU[3,4].

Therefore, environmentally friendly alternatives to TFA in peptide synthesis are sought to reduce the PFAS emission load from peptide production. The goal of this call is to identify a universal TFA-free protocol for the quick, clean, and safe release of synthetic peptides from the solid support following conventional Fmoc-SPPS (Fmoc = 9H-fluoren-9-yl) methoxycarbonyl-).

What potential solutions could be in scope?

Traditional protocols involve precipitating the crude peptide by adding antisolvents to the peptide TFA solution after cleavage. At this stage, alternative methods for capturing the raw peptide that avoid handling of potentially highly bioactive solids and flammable solvents could provide additional value (e.g., SPE, SEC). The solution would likely involve a combination of reagents, including, but not limited to, an appropriate acidic agent plus diluents that ensure resin swelling and peptide solubility plus nucleophilic scavenger to quench reactive intermediates. The following requirements should be met:

- Rapid and complete reaction around room temperature
- Clean conversion without the formation of unwanted by-products
- The proposed protocol is applicable to the common building blocks used in Fmoc-SPPS.

What potential solutions would be out of scope?

- Proposals aimed at using uncommon protecting groups to facilitate removal by non-acidic conditions.

- Proposals that imply the use of hazardous, highly corrosive, eco-toxic or commercially unavailable materials.
- Replacement of TFA as ion-pairing agent in eluent systems for peptide liquid chromatography is not intended to be subject of this program.

What benefits do we offer to you in exchange for having submitted a solution?

If your project is selected, you will have the opportunity to directly collaborate with the Peptide Teams in Medicinal Chemistry and Development NCE of Boehringer Ingelheim.

You can expect appropriate funding for the intended 12-month collaboration period. Your exact funding request should be outlined in your proposal. As a framework, we suggest that your initial funding request is structured by milestones and does not exceed USD 80,000.

The opportunity for a funded stay at Boehringer Ingelheim for technology exchange / training is potentially available.

Our collaboration agreement will provide full transparency about each partner's rights & obligations (including intellectual property rights). A final comprehensive report is due one month after the end of the grant period. As part of the agreement, you will be encouraged to publish your results in a peer-reviewed technical journal within 6 months after conclusion of the work. Each publication prepared in connection with the IU More Green Grant shall make acknowledgement in the following manner: "This manuscript was developed with the support of Boehringer Ingelheim's IU More Green Grant program, whose intent is to minimize the environmental footprint of future medicines through sustainable science, technology and innovation. Boehringer Ingelheim is a leading research-driven biopharmaceutical company that creates value through innovation in areas of high unmet medical need, and is working on breakthrough therapies that transform lives, today and for generations to come. Founded in 1885 and family-owned ever since, Boehringer Ingelheim takes a long-term perspective. Its commitment to contribute towards a healthier and more sustainable future is firmly anchored in our corporate philosophy since its founding and translated through the Sustainable Development for Generations (SD4G) framework. More than 52,000 employees serve over 130 markets in the three business areas, Human Pharma, Animal Health, and Biopharmaceutical Contract Manufacturing."

To maintain the highest degree of an open innovation environment, we plan to announce the winner(s) publicly and feature them on opnMe.com and our social media channels. We would guide you through this process, and we kindly ask for your upfront consent, in case our scientific jury selects your proposal.

What are the key success criteria on which we base our selection for the best answer?

We are seeking well-structured research collaboration proposals that outline:

- Model Peptides: Define appropriate model peptides that encompass a variety of protected sidechains and resin linkers. The proposal should detail the rationale for the chosen models and how they will aid in the investigation of potential replacements.
- Physicochemical Properties and Reactivity Data: Provide, if available, physicochemical properties and reactivity data that support the feasibility of the proposed surrogates for the intended purpose.
- Optimization Parameters: Identify the key parameters that need to be optimized in the cleavage/deprotection process with the proposed alternative reagents. This should include factors such as concentrations, composition of the cleavage cocktail, reaction time, temperature, among others.
- Analytical Methods: Propose robust and reliable analytical methods to monitor the effectiveness of the replacement.
- Outline how the proposed approach would help avoid PFAS and other hazardous reagents compared to conventional protocols.
- Your exact funding request should be outlined in your proposal based on a well-thought-through project. The project should be structured in milestones and planned with key decision points (clear Go/No-Go criteria). The funding request should not exceed USD 80,000.
- We will only consider project proposals which can be completed within 12 months or less. Within this period, you should be able to generate confirmation about your hypothesis based on predefined experimental milestones, as well as publishable results.
- Proven track record in the required field of expertise.

- Ability to implement the outlined solution as part of a scientific collaboration project with Boehringer Ingelheim including access to a laboratory.

What information should be included in your answer submission?

Please use our answer submission template to provide a 2–3-page non-confidential proposal (available for download on the following site).

If confidential data exists that would strengthen the proposal, please indicate that information is available to share under a Confidential Disclosure Agreement (CDA). If we find the non-confidential concept proposal sufficiently interesting, we will execute a CDA for confidential discussions.

Anticipated Project Phases or Project Plan

Phase 1	Please complete your submission by **May 28, 2024, 11:59 pm PST at** the very latest
Phase 2	Our review of all proposals will be completed by the end of July and scientists will be informed after that.
Phase 3	Start of discussions for the collaboration agreement in Q3/2024.

Submitting a collaboration proposal

- Check the outline of the opn2EXPERTS "Eco-friendly TFA-free solid-phase peptide synthesis" on opnMe.
- Alternatively, you may click the "Get Submission Template" banner to access the material transfer template.
- Follow the instructions to upload your submission document (requires login or registration).
- The upload allows you to attach additional application files if desired.
- You will be able to access your final submitted collaboration proposal in your personal dashboard and follow its review status.
- Please also visit the FAQ section on opnMe.com to learn more about our opn2EXPERTS program.

References

1. Freeling F., Björnsdotter M. K. Assessing the environmental occurrence of the anthropogenic contaminant trifluoroacetic acid (TFA). *Curr. Opin. in Green Sustain. Chem.* **2023**, 41, 100807. DOI:10.1016/j.cogsc.2023.100807
2. Cahill T. M. Increases in Trifluoroacetate Concentrations in Surface Waters over Two Decades. *Environ Sci Technol.* **2022**, 56(13):9428-9434. DOI: 10.1021/acs.est.2c01826. PubMed
3. Per- and polyfluoroalkyl substances (PFAS) - ECHA
4. Tyrrell N. D. A Proposal That Would Ban Manufacture, Supply, and Use of All Fluoropolymers and Most Fluorinated Reagents within the Entire EU. *Org. Process Res. Dev.* **2023**, 27, 8, 1422–1426. DOI:10.1021/acs.oprd.3c00199.
5. Chan W., White P. Fmoc Solid Phase Peptide Synthesis: A Practical Approach. *Oxford Academic* 16 December **1999**. DOI:10.1093/oso/9780199637256.001.0001.