CASE STUDY

The Trant team

University of Windsor, Canada https://trantteam.ca/

The Trant Team

Professor John Trant is an Associate Professor and Faculty of Science Research Chair at the University of Windsor in Canada. He established The Trant Team in 2016 at Windsor University. With 43 full time researchers (26 postdocs and staff scientists, 11 PhD candidates, 6 master students) the group is currently the largest medicinal chemistry research group in Canada.

The Trant team includes chemists, engineers, biologists, biochemists and materials scientists. Thanks to this wide set of skills, they are constantly looking for new ways of working, whether they are chemical or technical solutions.

The group focuses on applying synthetic organic chemistry tools to the challenges of biology, medicine and advanced materials.

Many of their main research projects are focused on developing new amino acids and carbohydrates, these are used to improve the pharmacokinetic properties of artificial oligosaccharides and peptides. Molecular stability in the human body is key to developing new drug candidates.

New chemical space

Over the last 50 years, the small molecule space has been intensively explored and there has now been a shift towards investigating peptides and small proteins. A large number of these peptide candidates contain unnatural amino acids as building blocks. A trend which is evidenced by the most famous peptide drugs of 2024; Semaglutide (Novo Nordisk), Tirzepatide (Eli Lilly) and PCSK9 inhibitor MK-0616 (Merck).

By improving the stability and morphology of these large biomolecules the duration of activity in the body is greatly enhanced, allowing the Trant Team to use them for further research towards:

- New drug candidates for immunological and anti-cancer applications
- New modalities of drug delivery
- As material for new medical diagnostics (for example, nano probes)

The Trant Team and Vapourtec

John Trant met Duncan Guthrie, Vapourtec's founder and director, through an online meeting. They started discussing some of Vapourtec pioneering work on using sub-stoichiometric amounts of amino acid during a synthesis without compromising the final purity, and that caught John's attention.

"And at that point, it was just like this literally changes absolutely everything. Vapourtec's Flow Technology allowing us to use stoichiometric, or even sub-stoichiometric amounts of an amino acid is a game breaking technology because the way we do peptide synthesis, whether it's automated or whether it's manual. We do both, and sometimes really special things, you want to do it manually, for better control, so you can't blame the machine if it goes wrong.

You are still throwing away so much of the material, and all of peptide synthesis has been designed around the fact that the amino acids are cheap. That's the underlying hypothesis that underlies the technology that we have built peptide synthesis around, but it is not always the case"

commented Prof Trant.

Duncan commented

"The main drive for this collaboration is to deepen the understanding of the kinetics of SPPS. This project is focused on exploring whether it is feasible to use an unnatural amino acid as the limiting reagent in SPPS. Showing the extent of its application is very exciting because it is applied science in action.

Going with the flow

One of the main issues the Trant team currently faces is with their peptide synthesis projects that involve unnatural amino acids.

At lab scale, peptides are usually synthesised using Solid-Phase Peptide Synthesis (SPPS). This process involves a repetition of coupling/deprotection reactions that allows the elongation of the peptide attached to a resin (solid support).

In batch mode, this process is very wasteful, not only in time, but in solvent and reagent (amino acid) consumption. With standard amino acids, scientists are resigned to use large excess, between 3 and 10 times the amount actually needed, but this becomes a major issue when unnatural amino acids are present in a sequence.

Typically, unnatural amino acids can be prepared in an up to 15 step synthetic route, which make them extremely precious. Using excess equivalent during a synthesis is highly undesirable.

Chemically speaking, Fmoc/tBu has not developed significantly since the 80's, however, recent implementations in continuous flow synthesis techniques are opening new opportunities. Fast-Flow SPPS offers a different way of synthesising peptides compared to batch methods.

The Vapourtec variable bed flow reactor (VBFR) technology inhibits the movement of resin beads while minimising the reactor volume throughout the whole synthesis. This ensures a unique interaction between the reagents and the static solid support, back mixing is eliminated and reaction by-products are removed.

By applying uniform heating, reaction kinetics are increased while aggregation events can be prevented, as heating prevents β-sheet structure formation.

These two key differences improve cycle efficiency and final crude purity of the target peptide. Additionally, the constraint of the resin in the variable bed reactor (VBFR) removes the randomness inherent in batch processes and ensures the desired peptide is targeted. The opportunity is then created to use a valuable unnatural amino acid as the limiting reagent.

The ongoing project

The Trant team has developed a series of unnatural amino acids as building blocks in peptides designed to treat diseases.

The current bottleneck is the synthesis of these novel unnatural amino acids. It takes weeks for a chemist to make them and purify them. If both time and reagent costs are added up, each scaffold could cost in excess of $15,000 per gram of unnatural amino acid due to the sometimes weeks of labour required.

This bottleneck becomes a twofold feasibility problem:

1. **Library of peptide analogues – if reagents are so costly, it would not be possible to synthesise permutations of a sequence until the desired properties are achieved. This may put a stop in the early discovery phase.**
2. **If a large excess of a very expensive amino acid is needed, it may not pass the process development stage as it would not be economically viable to be produced at larger scale.**

Since the team started working with FF-SPPS using the Peptide-Explorer, the data generated has been key to understand synthetic problems during a sequence.

The VBFR data enables the Team to understand when aggregations start, and with that information make chemical modifications to attenuate the extent of aggregation events, like using amino acids with a different side protective group.

The flow team, led by Dr Ezequiel Silva Nigenda noticed an improvement in purity which allow them in some cases to not perform a second purification of the peptide.

> *I'm a little bit surprised with the improved purity. We would expect the same levels the way we do the batch synthesis. We drive high conversion by using a massive excess of amino acid, and that works quite well.*
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> *As a general principle, when you are using more excess of any reagent in a reaction, you also increase the chances of side reactions occurring simultaneously. If we can decrease the amount of reagent that we need, we are also decreasing the opportunities for side reactions.*
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> *Most of us don't go digging into figuring out what all these minor impurities are. I think there's some of that going on. In theory, in a single pass approach the chemistry should be more controlled thanks to the VBFR and uniform heating. And this is what we hope this project will answer."*
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> **Ezequiel commented.**

Future of Fast Flow SPPS

For Prof Trant it is clear that peptides as new drug modalities have come to stay. For peptides to become more mainstream, scientists need to work on:

1. Improve cycle efficiency to increase purity and sustainability
2. Develop novel unnatural amino acids and new protective groups that can improve synthetic strategies
3. Make SPPS less wasteful

With more commercially available building blocks, new chemical strategies and an improved synthetic route, scientists will now access sequences that were difficult to make without coupling fragments.