SPECIALITY CHEMICALS MAGAZINE

Empowering highly potent peptide drug conjugate scale-up with flow chemistry

Youchu Wang, Ph.D.
Senior Vice President,
Head of API Early Phase Development,
WuXi STA, a WuXi AppTec company

In the last few years, we have seen increasing interest in and utilization of continuous processing for the development of novel pharmaceuticals, with the chief reason being its greater manufacturing efficiency and lower overall footprint. Continuous processing has also opened Pandora's box in helping overcome many scale-up issues that are unsolvable in conventional batch production setups.

In this article, we will look specifically at the development challenge faced when moving a peptide-drug conjugate (PDC) from pre-clinical (gram-scale) to phase I (kg-scale). New modality conjugations with HPAPIs and peptides – particularly for oncology targets – are now an increasingly large part of the discovery pipeline. Consequently, we will see many innovators and CDMOs looking to technologies like continuous manufacturing to meet the unique challenges of scale-up.

Experimental

Recently we worked with a biotech that was developing a new oncology drug and needed a new solution for the API manufacturing of a PDC, including a highly potent cytotoxin. The PDC can be successfully synthesized at the gram scale, but impurities emerged when it was scaled up to the kg level.

In order to achieve the assembly between the toxin-linker and peptide, the client chose the TBTU coupling reagent. In discovery-scale synthesis (25g scale), this assembly scheme worked well with a ~70% step yield. However, problems emerged during the process scale-up. The step yield dropped significantly and impurities started to appear.

Our team identified that the problem was caused by the decomposition of the activated ester intermediate of the toxin-linker-TBTU complex. While TBTU is a common assembly reagent in peptide (and oligonucleotide) conjugation, it does have limitations, primarily that the ester bond between TBTU and the linker is unstable, subject to nucleophilic attack. Thus, even when we carefully design a process, with all of the reagents and solvents selected to be non-nucleophilic, one factor still remains: the toxin-linker itself, which is likely to cause side reactions.

In this reaction in particular, the toxin-linker also had three nucleophilic hydroxyl groups (-OH), compounding the problems. This nucleophilic property resulted in intramolecular condensation when these alcohols attacked the TBTU ester (Figure 1) and this side reaction significantly dropped the yield and generated impurities.

This was not a problem in the gram-scale synthesis when the reaction time was short and the concentration of the toxin-linker-TBTU complex remained low. During the scaled-up manufacturing at kg-scale, however, with increased reaction time and local concentration, the side reaction between the complex itself significantly increased, posing impurity and yield challenges.

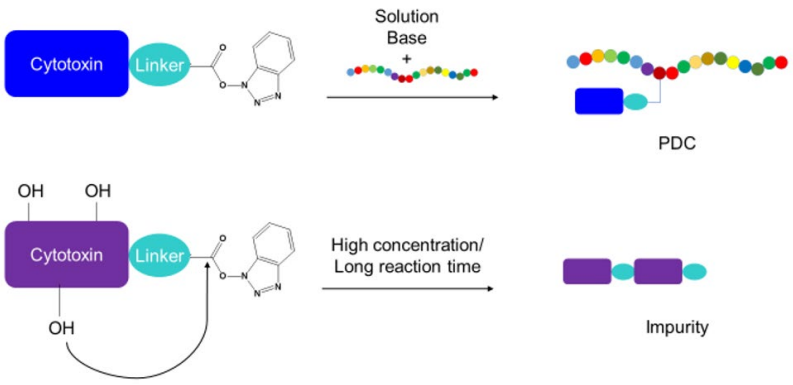

Figure 1: Toxin-linker and peptide conjugation via TBTU coupling (top); activated ester degradation due to an intracellular nucleophilic attack at high concentration or long reaction time (bottom)

Because of the special structure of the toxin, TBTU degradation was difficult to resolve in large-scale batch manufacturing. To overcome this challenge, our team at WuXi STA applied flow chemistry to avoid degradation conditions.

The starting materials were fed into one-way pipe reactors at precisely controlled time and in a precisely controlled quantity. The reactions were fully automated and took place in an enclosed system, minimizing human exposure to highly potent compounds.

After adjusting to the ideal reaction temperature, the toxin-linker-TBTU complex was generated continuously in the reaction stream (Figure 2, Reaction 1). The peptide was then fed into the system immediately and coupled with the toxin-linker-TBTU complex (Reaction 2).

The concentration of the complex was always limited to a low level by conjugating with the peptide in the flow mode before any side reaction could take place. Moreover, compared with the batch mode, the reaction temperature could be increased to 10°C, accelerating the reaction speed twofold.

As a result, 7 grams of crude PDC were manufactured every hour with a high yield of over 76%. With follow-up isolation and purification, multiple kilograms of the PDC API were delivered with more than 97% purity.

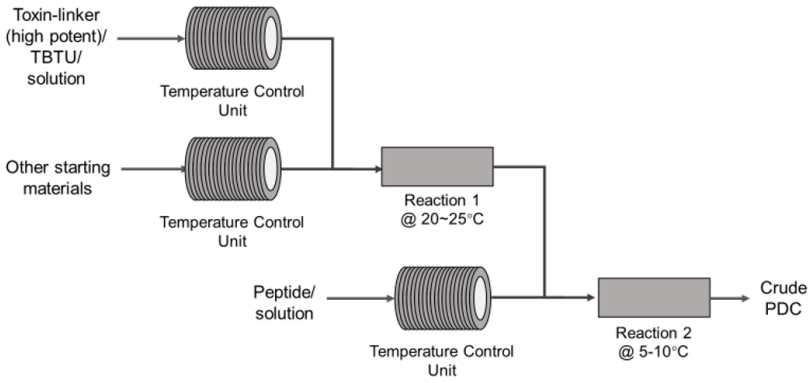

Figure 2: A demonstration of the flow chemistry process of PDC, TBTU, and toxin-linker coupling reaction.

Outlook

Flow chemistry technology was successfully employed in this project to address the scale-up challenge of this PDC drug. With the fully enclosed reactors and automated system, it minimized human exposure to highly potent compound.

The flow mode reduced the local concentration of the toxin-linker-TBTU complex. It also shortened the reaction time with increased temperature, so the condition for side reactions was avoided, showing unique advantages compared with the batch mode.

As PDC drugs involving powerful cytotoxins continue to thrive, it is foreseeable that flow chemistry will play an increasingly important role in enabling these challenging therapeutic modalities.