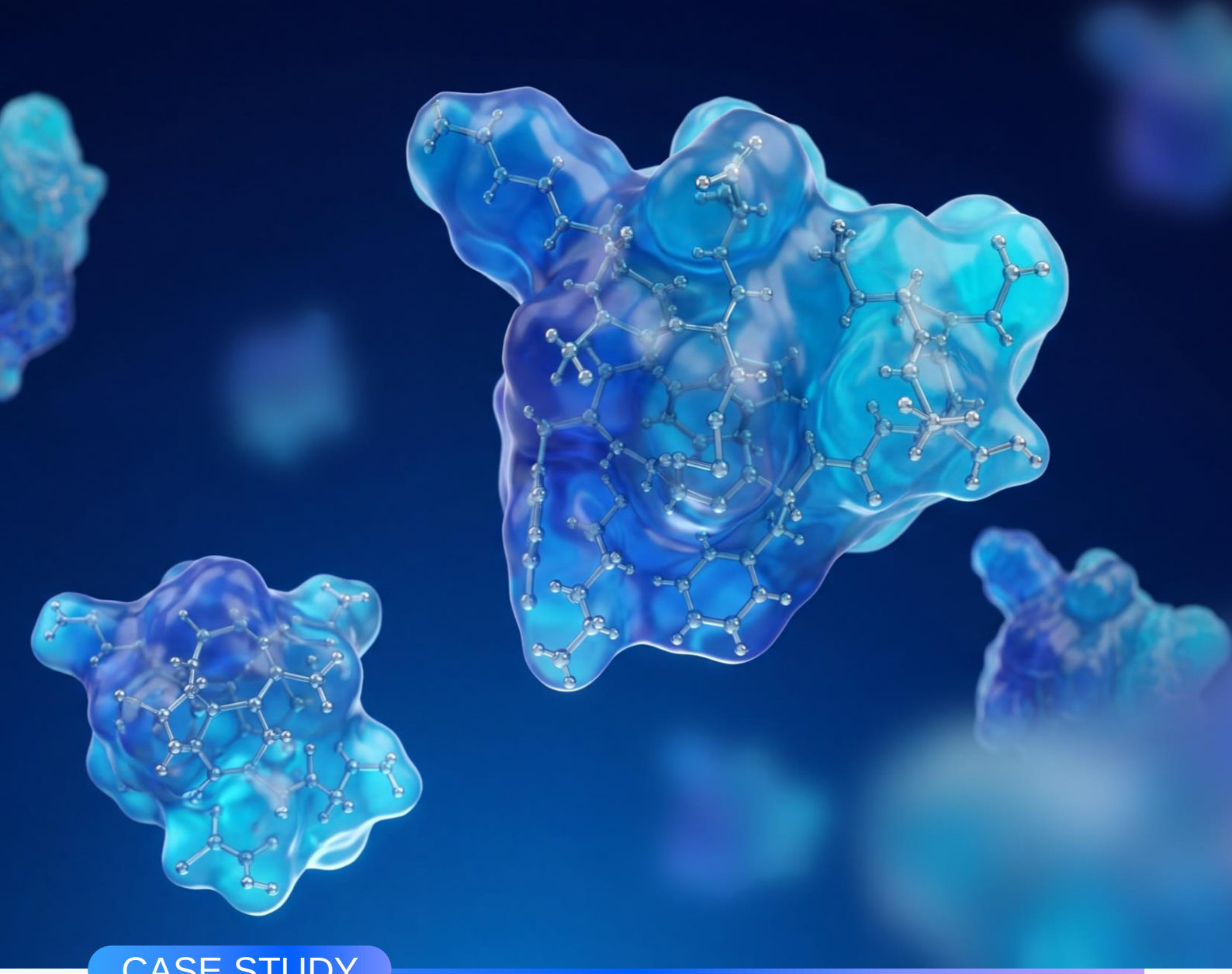


CASE STUDY

Optimization of Synthetic Process and Metal-Chelation Impurity Control Strategy for Complex Peptide-based RDCs

Cyclic Peptide, Radionuclide-drug Conjugate, Metal-chelation Impurity, Elemental Impurity, Single-use Freeze-drying Tray

PORTON

Introduction

Radionuclide-Drug Conjugates (RDCs) are core carriers for the integrated diagnosis and treatment of tumors, enabling simultaneous precise diagnosis and tumor killing. Among them, peptide-based RDCs have become an important direction for cancer treatment due to their high matching degree between half-life and radionuclides, excellent targeting, and good safety. To date, more than 400 RDC-related clinical trials have been registered worldwide. Despite their broad application prospects, R&D and manufacturing still face many core challenges:

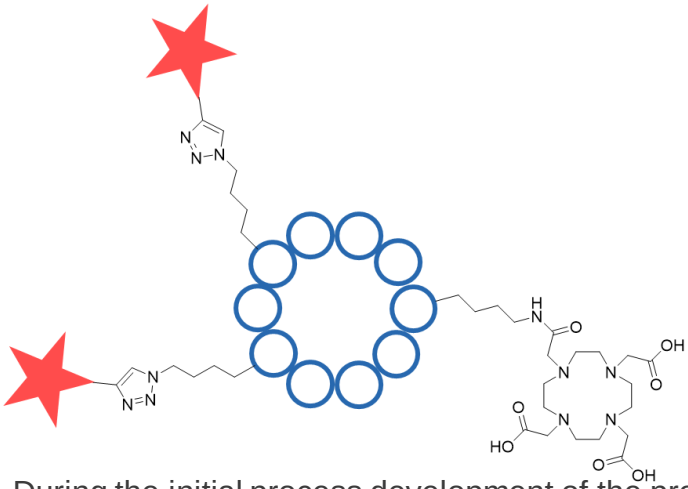
On the one hand, the construction of complex peptide structures for peptide-based RDCs requires the use of metal reagents, while core radionuclide chelating groups such as NOTA and DOTA are highly sensitive to metal ions. Residues of metal reagents and impurities are likely to affect radionuclide chelation efficiency, damage product quality, and compromise the clinical safety and effectiveness of drugs;

On the other hand, RDC R&D involves interdisciplinary integration and requires special production environment conditions. In addition, the short half-life of radionuclides poses challenges in transportation and stability control. In addition, the lack of clear non-clinical research guidelines has greatly raised the industry entry threshold.

In response, Porton, as a world-leading CDMO platform, has strategically positioned itself in the RDC CDMO service sector. With experience supporting dozens of nuclear drug clients in IND, NDA, and ANDA projects, Porton provides end-to-end CMC solutions ranging from process development to GMP production, effectively addressing industry challenges and facilitating the accelerated market launch of RDC drugs. Base on practical case studies, this paper focuses on the core pain points encountered during the R&D and production of peptide-based RDCs.

Background and Core Challenges

The main structure of the RDC molecule involved in this project is a cyclic peptide composed of ten amino acids, which bears one radionuclide-chelating DOTA group linked to one of the lysine side chains, and two special functional groups attached to the other two lysines via triazole rings. As the order of incorporation of these pendants might impact the physical properties of the intermediates, and the formation of triazole rings requires the use of metal catalysts, which poses challenges on the control of elemental impurities, careful synthetic route and process design are critical.



During the initial process development of the project, a feasibility evaluation of the medicinal chemistry route was conducted, and three core technical challenges were identified, which directly impacted product purity and production feasibility:

① Low CuAAC reaction efficiency: The solid-phase CuAAC reaction between the special functional groups and peptide chains exhibited numerous side reactions and poor selectivity, which could not be improved even after multiple rounds of optimization, resulting in low product purity;

② Defects in the cyclization process: To achieve solid-phase head-to-tail cyclization, glutamic acid was selected as the first amino acid, which was attached to the resin through its side chain, with the C-terminal carboxylic end protected in the form of an allyl ester. Upon the completion of stepwise elongation of the peptide backbone, the allyl ester was deprotected using Pd(PPh3)4 followed by the desired cyclization. Although this offered a shorter route to the cyclic product, this method suffered from low product purity as a result of the formation of capping impurities caused by residual piperidine, whose thorough elimination would require a solvent-consuming repeated deswelling-swelling washing process. The purging of residual Pd was also proved to be very difficult, leading to unacceptable Pd levels in the final product.

③ Risks of formation of metal-chelation impurities: Due to the metal-chelating properties of DOTA-functionality, the DOTA-bearing product is prone to form metal-chelation impurities. Owing to the large molecular weight differences between the product and the metal ions, even extremely low levels of metal ions would be translated into relatively high, intolerable product impurity levels. These metal-chelation impurities often lie close to the product peak in HPLC analysis and are difficult to be purged by means of chromatography. Stainless steel facilities and consumables, as well as glass vials, used during downstream processing such as purification, lyophilization and aliquoting, are potential sources metal ions, imposing risks of formation of metal-chelation impurities.

Optimization and Implementation of Synthetic Process

1. Cyclization Process Optimization: Route Adjustment from Solid-Phase to Liquid-Phase

To address the initial process bottlenecks, one of the core optimization strategies involved switching from the solid-phase cyclization to liquid-phase cyclization. With this change, the site of cyclization was no longer restricted, allowing for a comprehensive evaluation of different sites of cyclization. The optimal cyclization site was selected based on multiple factors, including degree of epimerization , cyclization efficiency, and the solubility of the corresponding fully protected linear peptide.

After selecting the appropriate site of cyclization and optimizing the reaction conditions, the degree of epimerization was successfully controlled to below 0.3%.Moreover, allyl-protection of the C-terminal carboxylic acid was no longer required for this liquid-phase cyclization process, and therefore the use of Pd-containing reagents for its deprotection was also eliminated.

2. CuAAC Reaction Optimization: Improving Reaction Efficiency and Purity

Conventional solution-phase CuAAC reaction conditions were found to be more advantageous than performing the reaction in solid phase, resulting in more efficient conversion and higher product purity. Since the reaction product was in the fully protected form, uses fully protected peptides as substrates, the product retained good hydrophobicity to allow repeated aqueous extraction for efficient Cu removal, at the same time preventing severe loss of product from its partition into the aqueous phase.

Key Impurity Control Strategies

1. Identification and Control of Cu(II)-Chelation Impurity

During the global deprotection of fully protected precursor to the target product with TFA, a new impurity came to our attention. This impurity, amounted to about 1%, eluted very close to the product. Extensive studies on purification conditions failed to purge this impurity to acceptable levels. LC-MS analysis indicated a molecular weight of M+61 for this impurity, and with further ICP-MS and spiking experiments, the identity of this impurity was confirmed to be the Cu(II)-chelation product.

In order to come up with a suitable control strategy for this impurity, the mechanism of formation of this impurity was studied. With reference to the distribution diagram (figure 1) of different chelation species between NOTA and Cu(II) ion across different pH from the literature Inorg. Chem. 2018, 57, 6, 3061–3072, on the study of the species distribution of different species, chelation took place even at pH 1, prompting that the formation of this impurity was 'inevitable' if copper was not thoroughly removed before the global deprotection step.

Based on this finding, two control strategies were applied:

- Cu(II) ions after the CuAAC step must be removed prior to cleavage to ensure their content is reduced below the target limit (10 ppm); Residual Cu level must be reduced to a level below 10 ppm.
- Addition of an appropriate scavenger during global deprotection to further remove residual Cu(II) ions.

As purification by means of crystallization was unlikely effective for peptide products, the primary Cu(II) removal strategies in this case involved aqueous extraction and slurry washing.

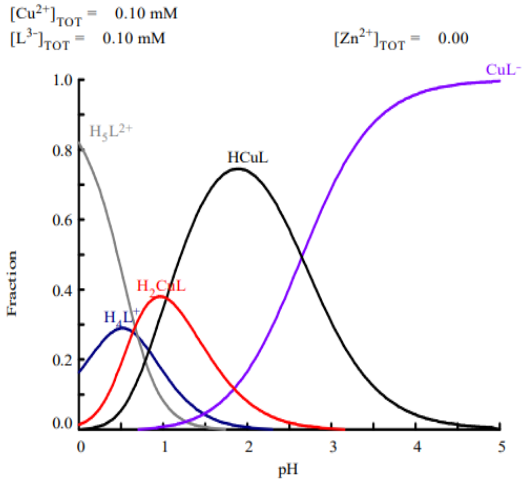


Figure 1. Inorg. Chem. 2018, 57, 6, 3061–3072

Among the various scavengers tested, sodium diethyldithiocarbamate gave the best performance. After 3–4 rounds of extraction, the Cu(II) ion content in the sample was reduced to below 100 ppm.

Subsequent slurry washing with an aqueous solution containing the same scavenger further lower the residual Cu(II) level to under 10 ppm.

For the global deprotection step, various sulfur-containing reagents were screened, but none gave satisfactory results. After multiple rounds of experiments, DOTA-$(COOH)_4$ was chosen as the optimal scavenger, successfully lowering the Cu(II) ion content to an even lower level.

2. Control of Other Metal Ion Chelating Impurities

To avoid metal ion contamination from stainless steel lyophilization trays during the lyophilization of purified fractions, disposable lyophilization trays made of polytetrafluoroethylene (PTFE) were used.

In order to eliminate the risk of the formation of metal-chelation impurities introduced from the direct contact between purified fractions and stainless steel lyophilization trays during lyophilization, single-use PTFE trays were used.

During the study of sample aliquoting into vials through product reconstitution and re-lyophilization, the DOTA-bearing product would induce leaching of metal ions in glass into the product solution, resulting in the formation various metal-chelation impurities. This issue was particularly severe with amber vials. Eventually, through screening of vials from different suppliers and implementing strict aliquoting conditions, impact on the product quality was reduced to an acceptable and minimal level.

When evaluating vials for the reconstitution and re-lyophilization of the lyophilized product, it was found that the sample solution would chelate with metal ions leached from the vials, leading to excessive levels of related metal ion chelating impurities. This issue was particularly severe with iron ion leaching from brown vials. Subsequently, the problem of excessive impurities was resolved by screening suitable vial materials.

Conclusion

The above RDC drug precursor CMC service project showcases strategies to cope with the core pain points in the R&D and production of peptide-based RDCs, focusing on overcoming key challenges such as the construction of complex peptide structures, and control of elemental impurities and their derived chelation impurities. With comprehensive and customized technical solutions, Porton achieved a breakthrough from laboratory gram-scale to hundred gram-scale GMP production in just 7 months through the development and optimization of new routes and flexible adjustment of CMC strategies. The final product purity reached over 98%, and the project was successfully delivered.

This project fully demonstrates the strength of Porton's one-stop RDC service platform, excellent project management capabilities, and efficient production and operation. Leveraging its comprehensive end-to-end RDC CDMO service capabilities, Porton has established an integrated service platform covering radionuclide-small molecule drugs, liquid-phase/solid-phase-synthesized radionuclide-macromolecular drugs, and radionulide-biomacromolecular drugs. Porton offers customized and integrated CMC services to help global clients expedite the advancement of RDC and cyclic peptide projects, enabling earlier access to good medicines.

About Porton TIDES CDMO Service Platform

Porton TIDES CDMO Service Platform has established multiple R&D centers and GMP production bases in Shanghai and Chongqing, China, aiming to provide end-to-end integrated CDMO solutions for peptide and oligonucleotide products for global pharmaceutical companies and new drug R&D centers, supporting different development stages from preclinical to commercial launch.

Porton's CDMO service covers process development & optimization, toxicology batch and clinical-stage sample production, structural characterization, analytical method development and validation, in-process control and product release testing, ICH-compliant stability studies, and global new drug filing, as well as other one-stop CMC services, committed to enabling earlier access to good medicines.

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