API Syntheses: Transfer from Lab Scale to GMP Manufacture

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Abstract

Once a potential drug candidate is about to leave lab scale and enter preclinical or clinical studies, the need for larger scale synthesis and eventually GMP compliant manufacture arises. Broad chemical and regulatory experience is needed together with a solid infrastructure in order to guarantee successful upscaling and production of an API (Active Pharmaceutical Ingredient) suitable for clinical trials. Even though the possibility for GMP compliant manufacture on larger scale is mostly lacking in small companies, there is a gap that even the big ones often find difficult to fill: the first 10 g to 10 kg of API for up to clinical phase II. ORPEGEN Peptide Chemicals has specialized in closing this gap between lab scale and routine industrial production, providing contract manufacturing services for peptides and small molecules including full know-how transfer to the customers. Two recent examples are given from the company's 28 years history.

Example 1: Chlorine e6 – a small molecule for photodynamic therapy

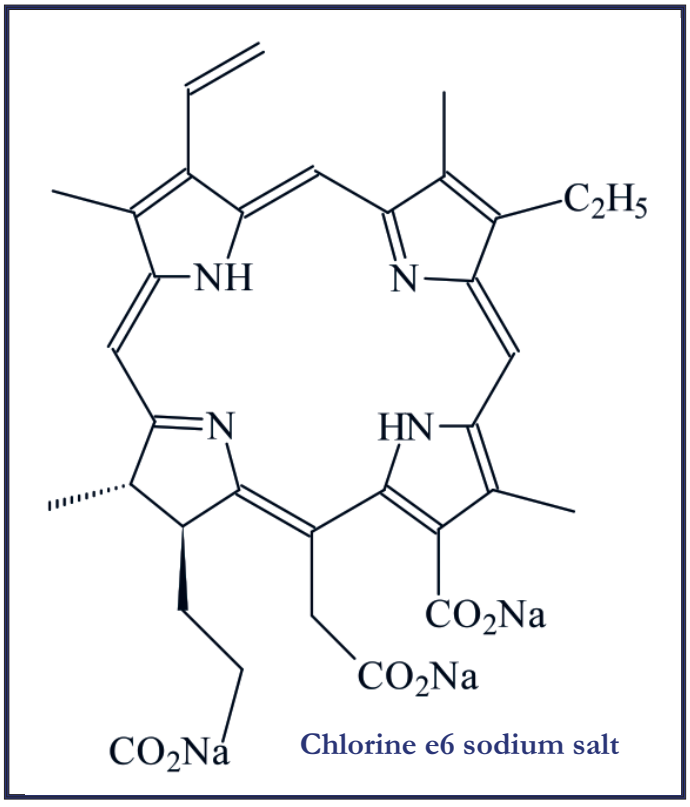
Photodynamic therapy, or PDT for short, is a special, most advanced form of cancer therapy using light together with a substance known as a photosensitizer, which accumulates selectively in tumour tissue and is then irradiated with light of a wavelength specific for that substance. Exposure of the photosensitizer to light results in the formation of a substance toxic to cells and thereby destroying the tumour cells.

With the advent of improved photosensitizers and the availability of information from well-controlled, randomized phase III clinical trials, PDT has gained a niche in the comprehensive cancer care regime.

It has been shown that molecules with a chlorin-type structure are able to generate singlet oxygen with a high quantum yield. Chlorins also exhibit a large extinction coefficient in the red region of the visible spectrum that is one order of magnitude higher than the corresponding extinction coefficient for porphyrins.

Of particular interest among the evaluated chlorin is the Chlorin e6 (Ce6). Ce6 has improved efficacy and has decreased side effects compared to first generation photosensitizers from hematoporphyrin derivatives.

Thus, the Ce6 sodium salt has a great application potential in fluorescence diagnosis and photodynamic therapy. We further investigated the fluorescence and photodynamic activity of Ce6, which was prepared using pharmaceutically accepted methods of preparation (cGMP) at ORPEGEN Peptide Chemicals GmbH, Heidelberg, Germany.



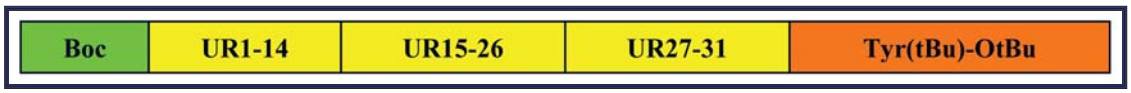
Chlorine e6 sodium salt

Example 2: Urodilatin – a cyclic 32mer peptide increasing diuresis

Urodilatin is a hormone that causes diuresis through increasing renal blood flow. It is secreted in response to increased mean arterial pressure and increased blood volume from the cells of the distal tubule and collecting duct. It is important in oliguric patients (those with acute or chronic renal failure) as it lowers serum creatinine and increases urine output.

Urodilatin must be synthesized in fragments that are condensed in solution and cyclized afterwards – an extremely demanding synthesis and purification.

The polypeptide sequence is TAPRSLRRSSCFGGRMDRIGAQSGLGCNSFRY with a disulfide bridge between Cys^{11} and Cys^{27}. Three peptide fragments are prepared by SPPS on 2-Cl-Trityl-resin and cleaved by dilute TFA thus preserving the side chain protecting groups. Since all protected fragments have a free carboxy terminus, the C-terminal fragment must be converted into a tert.-butyl ester before starting the first condensation reaction. Reagents used for this reaction are only available on research scale, but the problem is solved by synthesizing the fragment 27-31 (instead of 27-32) and coupling the last amino acid tyrosine in it's di-tert.-butylated form (H-Tyr(tBu)-OtBu) in solution, an in-house manufacturing specialty of ORPEGEN Peptide Chemicals. Overall condensation is done by coupling the fully protected fragments in solution according to the following scheme:



HPLC-chromatogram of Urodilatin production batch

After final side chain deprotection, the peptide is cyclised in solution and purified by two successive HPLC runs in different buffer system, finally yielding the API in a purity of > 99 % (rp-HPLC, 220 nm).