DOCTORAL THESIS

Combining Dipyrrin Formation with Solid-phase Peptide Synthesis: Practical Approaches to Synthesize Fluorescent Peptides and Multi-functional Cyclopeptides

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ABSTRACT

Over a century, peptides have been used as drugs for treating various diseases. In recent decades, along with the development of structural biology, peptides have even been regarded as promising modulators for protein-protein interactions (PPIs) that play vital roles in all biological processes. Fluorescent peptides are powerful tools in chemical biology, which are widely applied for diagnoses and fundamental studies. As the most established protocol for synthesizing peptide derivatives in both laboratory and industry, solid-phase peptide synthesis (SPPS) shows significant advantage that the desired products can be obtained with no more than single time chromatographic purification, while all excess reagents/reactants and the side products in the solution phase can be removed by simple wash.

Dipyrrins and their boron complexes BODIPYs have attracted lots of research attentions and efforts for their potential applications. As a series of bright and tunable fluorescent molecules, BODIPYs have been used as fluorescent dye for decades. Although dipyrrins are not fluorescent molecule, they serve as ligands for various fluorescent metal complexes, and the turn-on process upon complexation give their potential for metal sensing. A functionalized dipyrrin/BODIPY, which requires multi-step reaction and purification, is thought to be necessary for obtaining its corresponding peptide conjugate.

In this thesis, the syntheses of dipyrrin/BODIPY moieties were strategically and innovatively combined with the procedure of SPPS rather than conducted independently; dipyrrin/BODIPY-peptide conjugates were obtained with significantly lower workload. By using simple aldehydes/orthoesters/acyl chlorides and pyrroles as building blocks, the fluorescent BODIPY-peptide conjugates and

multifunctional dipyrrin/BODIPY-based cyclopeptides were able to be obtained by performing only single time chromatographic purification.

In Chapter One, the research background and significance about peptides, solid-phase peptide synthesis, fluorescent peptides, as well as dipyrrins and BODIPYs are introduced.

In Chapter Two, my first research project is described that provides a novel synthetic methodology to *in situ* construct dipyrrin on solid support directly. By using simple aldehydes and pyrroles, various dipyrrins can be constructed on diverse peptides, which include all commonly used amino acids, during SPPS. The post-cleavage dipyrrin-peptide conjugates, which also include all commonly used amino acids, can be converted into fluorescent BODIPY-peptide conjugates which are potential bio-targeting fluorescent probes. Specially, one of BODIPY-peptide conjugates, **BODIPY$_1$-Pep$_4$**, showing similar bioactivity as other EBNA1-targetting fluorescent probes reported in our previous research, are promising fluorescent probe and photodynamic therapy agent for EBV infection.

In Chapter Three, my second project is described where a novel peptide macrocyclization methodology can be achieved by constructing dipyrrin on resin intramolecularly. The *meso* position of formed dipyrrin cyclopeptides can be diversified by using different commercially available orthoesters, acyl chlorides and aldehydes. The SPPS can be even continued on the *meso* position via specific functional group. The products showed higher protease resistance than their linear counterparts, and showed various potential applications. The dipyrrin cRGD peptide **cDP$_1$-Pep$_{14}$** showed good binding affinity with integrin $\alpha_v\beta_3$, and its corresponding BODIPY **cBODIPY$_1$-Pep$_{14}$** performed as a promising fluorescent for bladder cancer cell lines. Moreover, the dipyrrin cGHK peptide **cDP$_1$-Pep$_{11}$** showed sensitive and selective response toward zinc ion *in vitro*.

In Chapter Four, all detail of experiments, including the chemical syntheses, biological experiments and computational experiments are described. Moreover, the NMR spectra, HPLC chromatographs and HRMS spectra of synthesized compounds are also presented on Appendix.

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