

## 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 dipyrin/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 dipyrins and BODIPYs are introduced.

In Chapter Two, my first research project is described that provides a novel synthetic methodology to *in situ* construct dipyrin on solid support directly. By using simple aldehydes and pyrroles, various dipyrins can be constructed on diverse peptides, which include all commonly used amino acids, during SPPS. The post-cleavage dipyrin-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<sub>1</sub>-Pep<sub>4</sub>**, showing similar bioactivity as other EBNA1-targeting 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 dipyrin on resin intramolecularly. The *meso* position of formed dipyrin 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 dipyrin cRGD peptide **cDP<sub>1</sub>-Pep<sub>14</sub>** showed good binding affinity with integrin  $\alpha_v\beta_3$ , and its corresponding BODIPY **cBODIPY<sub>1</sub>-Pep<sub>14</sub>** performed as a promising fluorescent for bladder cancer cell lines. Moreover, the dipyrin cGHK peptide **cDP<sub>1</sub>-Pep<sub>11</sub>** 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.

# Table of Contents

DECLARATION .....	i
ABSTRACT .....	ii
ACKNOWLEDGEMENTS .....	v
Table of Contents .....	vii
List of Tables .....	x
List of Figures .....	xi
List of Abbreviations and Symbols .....	xix
Chapter One. Introduction .....	1
1.1 Peptides and their application .....	2
1.1.1 Amino acids .....	2
1.1.2 Peptide .....	4
1.1.3 Biological application of peptide .....	7
1.2 Solid-phase peptide synthesis .....	10
1.3 Fluorescent peptides as bioprobes .....	17
1.4 Dipyrin and BODIPY .....	20
1.5 Objectives of this thesis .....	26
1.6 Reference .....	27
Chapter Two. Solid-phase fluorescent BODIPY-peptide synthesis via <i>in situ</i> dipyrin construction .....	36
2.1 Introduction .....	37

2.1.1	Current synthetic methodology for BODIPY-peptide conjugates.....	37
2.1.2	Epstein–Barr virus (EBV) and Epstein-Barr nuclear antigen 1 (EBNA1).....	39
2.1.3	Photodynamic therapy (PDT) .....	44
2.2	Result and discussion.....	48
2.2.1	General Design.....	48
2.2.2	<i>In-situ</i> construction of different dipyrrens on YFMVF peptide.....	51
2.2.3	<i>In-situ</i> construction of dipyrren on different peptides .....	53
2.2.4	Conversion of dipyrren-peptide conjugates into BODIPY counterparts .....	54
2.2.5	<b>BODIPY<sub>1</sub>-Pep<sub>4</sub></b> as EBNA1-targeting fluorescent probe and PDT agent .....	57
2.3	Conclusion.....	61
2.4	Reference.....	62
Chapter Three. Direct formation of multifunctional dipyrren-based cyclopeptide .....		71
3.1	Introduction .....	72
3.1.1	The disadvantages of linear peptides .....	72
3.1.2	Design and synthesis of cyclopeptides.....	73
3.1.3	Integrin $\alpha_v\beta_3$ . .....	83
3.2	Result and discussion.....	88
3.2.1	General design.....	88

3.2.2	Synthesis of dipyrin cyclopeptide .....	88
3.2.3	Dipyrin/BODIPY-based cRGD peptide as $\alpha_v\beta_3$ probe .....	93
3.2.4	Dipyrin-based cGHK peptide as selective zinc sensor .....	99
3.3	Conclusion.....	105
3.4	Reference.....	106
Chapter Four. Experimental.....		114
4.1	Chemistry .....	115
4.2	Biological experiments .....	147
4.3	Computational experiments .....	152
4.4	Reference.....	153
Appendix .....		155
	NMR spectrum of synthesized compounds .....	156
	HPLC chromatographs and MALDI-TOF HRMS spectra of products .....	201
CURRICULAM VITAE .....		273

## List of Tables

<b>Table 2.1</b> Expression of EBV viral proteins in different latency programs. ....	40
<b>Table 2.2</b> Photophysical property of BODIPY <sub>n</sub> -Pep <sub>1</sub> in DMSO.....	56
<b>Table 3.1</b> The estimated docking energies of <b>Pep</b> <sub>13-16</sub> and <b>cDP</b> <sub>1</sub> - <b>Pep</b> <sub>13-16</sub> with $\alpha_v\beta_3$ protein (PDB: 4MMY) with the residues that exist H-bond interactions with the docked position of respective compound.....	96
<b>Table 3.2</b> The photophysical property of <b>cBODIPY</b> <sub>1</sub> - <b>Pep</b> <sub>13</sub> and <b>cBODIPY</b> <sub>1</sub> - <b>Pep</b> <sub>14</sub> in HEPES buffer. ....	98
<b>Table 3.3</b> The photophysical property of <b>cDP</b> <sub>1</sub> - <b>Pep</b> <sub>11</sub> , <b>cDP</b> <sub>1</sub> - <b>Pep</b> <sub>12</sub> and <b>DP</b> <sub>18</sub> - <b>Pep</b> <sub>18</sub> in HEPES buffer with saturated zinc ion. ....	102
<b>Table 4.1</b> Gradient A for analytical HPLC .....	116
<b>Table 4.2</b> Gradient B for analytical HPLC.....	116
<b>Table 4.3</b> Gradient C for analytical HPLC.....	116
<b>Table 4.4</b> The chemical shift of $\alpha$ H/NH and corresponding coupling constant of RGD motif of <b>Pep</b> <sub>13-16</sub> , <b>cDP</b> <sub>1</sub> - <b>Pep</b> <sub>13-16</sub> .....	146

## List of Figures

<b>Figure 1.1</b> The structure, name, abbreviation (3-letter and 1-letter) of 20 standard proteinogenic amino acids. The backbones of amine acids are in blue, while their side chains are in black. ....	2
<b>Figure 1.2</b> Examples of non-proteinogenic amino acids. ....	3
<b>Figure 1.3</b> The example for the writing of peptide sequence. ....	4
<b>Figure 1.4</b> The different secondary structure of peptide. a) $\alpha$ -helix; b, c) parallel/antiparallel $\beta$ -sheet. ....	5
<b>Figure 1.5</b> The dihedral angle $\phi$ , $\psi$ and $\omega$ in peptide backbone. ....	6
<b>Figure 1.6</b> The Ramachandran plot for describing the secondary structure of peptides and proteins. ....	7
<b>Figure 1.7</b> The critical peptide sequence near interface of PPIs may be identified as modulators. ....	9
<b>Figure 1.8</b> Workflow of Fmoc SPPS. ....	10
<b>Figure 1.9</b> Structure and abbreviation of amino acid building blocks used in Fmoc SPPS, the protecting group on N-terminal and side chain are highlighted in blue and red, respectively. ....	12
<b>Figure 1.10</b> Examples of solid support for SPPS. ....	13
<b>Figure 1.11</b> The synthetic routes for DOTA-peptide conjugates. ....	15
<b>Figure 1.12</b> Jablonski diagram for the fluorescence. ....	17
<b>Figure 1.13</b> Examples of organic fluorescent molecules. ....	18

<b>Figure 1.14</b> The structure, numbering system and relationship of dipyrromethane, dipyrin and BODIPY. ....	20
<b>Figure 1.15</b> The synthetic methods of dipyrin. ....	21
<b>Figure 1.16</b> The post-functionalization of the BODIPY core.....	22
<b>Figure 1.17</b> The structure-property relationship of BODIPYs. ....	23
<b>Figure 1.18</b> The examples of dipyrin metal (zinc/cobalt) complexes.....	25
<b>Figure 2.1</b> Various approaches for synthesizing fluorescent BODIPY-peptide conjugates. A) Solution phase conjugation of fluorescent BODIPY dyes on post-SPPS peptides requiring multistep transformations and purifications. B) Pre-functionalized BODIPY-bearing unnatural amino acids were used as building blocks during SPPS.....	38
<b>Figure 2.2</b> The structure of Epstein-Barr virus. ....	39
<b>Figure 2.3</b> Location and function of EBNA1 domains.....	41
<b>Figure 2.4</b> EBNA1 DD/DBD domains bind with OriP of DNA. The green and orange represent the DD domain of two identical monomers, while pink represents the DBD domain for each. ....	42
<b>Figure 2.5</b> The vital short peptide Y <sub>561</sub> FMVG <sub>565</sub> was identified from the PPIs between two EBNA1 monomers.....	43
<b>Figure 2.6</b> Reported peptide-based EBNA1 dimerization domain inhibitors/probes. ....	44
<b>Figure 2.7</b> The Jablonski diagram for ROS generation.....	44
<b>Figure 2.8</b> The examples of photosensitizers.....	46

<b>Figure 2.9</b> The strategies for enhancing the singlet oxygen quantum yield of BODIPYs.....	47
<b>Figure 2.10</b> <i>On-resin</i> dipyrin construction follow by post-cleavage boron complexation to give the BODIPY-peptide conjugates with a single chromatographic purification step. ....	48
<b>Figure 2.11</b> The trial of dipyrin formation on peptide YFMVF ( <b>Pep<sub>1</sub></b> ).....	49
<b>Figure 2.12</b> Directly construction of dipyrin on resin-bound <b>Pep<sub>1</sub></b> . <b>A.</b> The samples of resin-bound peptide were taken, cleaved, precipitated and then monitored by HPLC and ESI-MS; From top to bottom: unmodified peptide (black), aldehyde-peptide conjugate (blue), dipyrin-peptide conjugate <b>DP<sub>1</sub>-Pep<sub>1</sub></b> (red); From left to right: HPLC of crude samples, UV-Vis spectrum at peak on HPLC, ESI mass of crude samples; <b>B.</b> Comparison of <sup>1</sup> H NMR of <b>Pep<sub>1</sub></b> and <b>DP<sub>1</sub>-Pep<sub>1</sub></b> . ....	50
<b>Figure 2.13</b> <i>In situ</i> construction of dipyrin derivatives on <b>Pep<sub>1</sub></b> . Condition: a) aldehyde-containing carboxylic acid, PyBOP, DIPEA, DMF, 3 h. b) pyrrole derivatives, BF <sub>3</sub> ·OEt <sub>2</sub> , r.t., DMF, overnight; c) DDQ, 1 h, DCM; d) TFA/TIPS/H <sub>2</sub> O, v/v/v, 95/2.5/2.5, r.t., 2 h. <sup>a</sup> Absolutely isolated yields compare with substitution values of resin-bound peptide. <sup>b</sup> Relative yield compare with isolated yields of unmodified peptide. <sup>c</sup> No reaction. <sup>d</sup> Desired products were detected by LC-MS, but too less to separate. ....	51
<b>Figure 2.14</b> The trial of dipyrin formation on peptide YFMVF ( <b>Pep<sub>1</sub></b> ) that used 5-formyl-2,4-dimethyl-3-pyrrolicarboxylic acid (FDMPA) as building block. The FDMPA can be regard as an aldehyde building block to form <b>DP<sub>14</sub></b> (a tripyrin example) or an α-formylpyrrole building block to form <b>DP<sub>15</sub></b> (an asymmetry example). ....	52

**Figure 2.15** On-resin construction of **DP<sub>1</sub>** on different peptides. <sup>a</sup> Absolute isolated yield compared with substitution value of the resin-bound peptide. <sup>b</sup> Relative yield compared with the isolated yield of the unmodified peptide.....53

**Figure 2.16** Boron complexation for post-cleavage dipyrin-peptide conjugates. <sup>a</sup> Purified dipyrin conjugate was used, isolated yields compare with corresponding dipyrin conjugate. <sup>b</sup> Crude dipyrin conjugates were used, isolated yields compare with substitution values of resin-bound peptide. <sup>c</sup> EDT was used as reductant. ....54

**Figure 2.17** The fluorescence of 10 μM BODIPY-peptide conjugates in DMSO under 365 nm UV light, with their normalized emission spectrum. ....56

**Figure 2.18** The comparison of <sup>1</sup>H-NMR between **DP<sub>1</sub>-Pep<sub>1</sub>** and **BODIPY<sub>1</sub>-Pep<sub>1</sub>**. .....56

**Figure 2.19** Fluorescent titration of BSA and EBNA1 protein toward **BODIPY<sub>1</sub>-Pep<sub>4</sub>**. EBNA1 showed around 6 times enhancement over titration, while BSA showed only 3 times. Which indicated **BODIPY<sub>1</sub>-Pep<sub>4</sub>** is selective response to EBNA1 protein. ....57

**Figure 2.20** The confocal imaging of **BODIPY<sub>1</sub>-Pep<sub>4</sub>** toward HeLa and C666. The green fluorescent signal (from BODIPY) uptake in nucleus (blue) of C666 (EBNA positive cell line) while not in nucleus of HeLa (EBNA negative cell line).....58

**Figure 2.21** Profiles of the emission intensity of the **BODIPY<sub>1</sub>-Pep<sub>4</sub>** and nuclear blue were plotted along the red arrow A. C666, B. HeLa. The signal of **BODIPY<sub>1</sub>-Pep<sub>4</sub>** and nuclear blue overlapped well in C666 cell line (EBNA positive) but not in HeLa cell line (EBNA negative). .....59

**Figure 2.22** The singlet oxygen quantum yield measurement. The solution of ABDA (50 μM) with rose Bengal (A, 10 μM) or **BODIPY<sub>1</sub>-Pep<sub>4</sub>** (B, 10 μM) were irradiated, and the absorption spectrum was recorded at 0, 1, 3, 5, 7, 9, 11 min. The decreasing

rates of absorption intensity of ABDA were used for calculation, where the singlet oxygen quantum yield of rose Bengal is 0.75; C) Singlet oxygen signal was found on emission spectrum of **BODIPY<sub>1</sub>-Pep<sub>4</sub>**; D) Comparison of dark/light cytotoxicity of **BODIPY<sub>1</sub>-Pep<sub>4</sub>** toward C666 cell line. Light toxicity was significantly higher than dark toxicity, which showed the PDT effect. ....60

**Figure 3.1** The disadvantages of linear peptide. ....72

**Figure 3.2** Different types of cyclopeptide according to the location of cyclic linker. ....73

**Figure 3.3** On-resin peptide macrocyclization and in-solution peptide macrocyclization. ....74

**Figure 3.4** One-component stapling and two-component stapling. ....75

**Figure 3.5** One-component stapling that conducted between natural amino acids. Disulfide bond formation and lactonization. ....76

**Figure 3.6** One-component stapling via C-H bond activation between tryptophan and halogenated tyrosine or phenylalanine. ....76

**Figure 3.7** One-component stapling via transitional metal catalysis. CuAAC and RCM reaction. ....77

**Figure 3.8** One-component stapling via Diels-Alder reaction. ....77

**Figure 3.9** Potential side reaction of two-component stapling, di-substituted side reaction. ....78

**Figure 3.10** Two-component stapling via formation of thioester. ....79

**Figure 3.11** Examples of two-component stapling with the introduction of functional moieties. ....80

<b>Figure 3.12</b> Two-component stapling between lysine, tyrosine/arginine and formaldehyde. ....	80
<b>Figure 3.13</b> Two-component stapling via lactonization. ....	81
<b>Figure 3.14</b> Two-component stapling via CuAAC. ....	82
<b>Figure 3.15</b> The design of $\beta$ -hairpin for mimicking the $\beta$ -sheet conformation. The examples of turn templates were highlight in green. ....	83
<b>Figure 3.16</b> The role of integrin in cell-ECM adhesion. The ECM-embed glycoproteins activate the integrin by binding, and active integrins are then able to bind with downstream protein in cell. ....	84
<b>Figure 3.17</b> The integrins with different $\alpha$ and $\beta$ subunits and their functions. ....	84
<b>Figure 3.18</b> The RGD motif plays vital role on the PPI between fibronectin and $\alpha_v\beta_3$ . ....	85
<b>Figure 3.19</b> The examples of $\alpha_v\beta_3$ -targeting cRGD peptides. ....	86
<b>Figure 3.20</b> Synthetic route of cyclo(-RGDfK-) conjugates by SPPS. ....	87
<b>Figure 3.21</b> Formation of dipyrin-based cyclopeptide. Percent conversion was determined by HPLC. The meso-position (red part in structure) was introduced by different condition: <sup>a</sup> Orthoester, POCl <sub>3</sub> , DCM, 12 h; <sup>b</sup> Acyl chloride, DCM, 12 h; <sup>c</sup> 1) aldehyde, BF <sub>3</sub> ·OEt <sub>2</sub> , DMF, 12 h; 2) DDQ, DCM, 1 h; * Further reactions were carried out after formation of dipyrin to yield corresponding products. ....	89
<b>Figure 3.22</b> The synthetic route of dipyrin cyclopeptide synthesis on GHK peptide. ....	89
<b>Figure 3.23</b> The synthetic route of synthesis of dipyrin-based bicyclic peptide <b>cDP<sub>1</sub>-Pep<sub>12</sub></b> and <b>cDP<sub>1</sub>-Pep<sub>13-16</sub></b> . ....	91

<b>Figure 3.24</b> The synthetic route of synthesis of dipyrin-based bicyclic peptide <b>bcDP-Pep<sub>17</sub></b> . .....	91
<b>Figure 3.25</b> The synthetic route of synthesis of dipyrin-bis-peptide conjugate <b>DP<sub>18</sub>-bis(Pep<sub>18</sub>)</b> . .....	92
<b>Figure 3.26</b> The synthetic route of synthesis of liner dipyrin-GHK peptide conjugate <b>DP<sub>19</sub>-Pep<sub>11</sub></b> .....	93
<b>Figure 3.27</b> In vitro trypsin resistance assays for cyclic <b>cDP<sub>1</sub>-Pep<sub>13</sub></b> versus linear <b>Pep<sub>13</sub></b> at 37 °C. ....	94
<b>Figure 3.28</b> The CD spectrum of 50 μM linear peptides <b>Pep<sub>13-16</sub></b> and dipyrin cyclopeptides <b>cDP<sub>1</sub>-Pep<sub>13-16</sub></b> in PBS 7.4.....	95
<b>Figure 3.29</b> α <sub>v</sub> β <sub>3</sub> binding assay of dipyrin cyclopeptides <b>cDP<sub>1</sub>-Pep<sub>13-16</sub></b> , linear peptide <b>Pep<sub>14</sub></b> , and the positive control cilengtide.....	96
<b>Figure 3.30</b> The docked structure of <b>cDP<sub>1</sub>-Pep<sub>14</sub></b> with α <sub>v</sub> β <sub>3</sub> (PDB: 4MMY) with ΔG = −8.8 kcal/mol. The dipyrin connector are shown in magenta while the peptide is shown in green. Fibronectin were overlaid in yellowish white with transparency as a comparison.....	97
<b>Figure 3.31</b> Boron complexation of <b>cDP<sub>1</sub>-Pep<sub>13</sub></b> and <b>cDP<sub>1</sub>-Pep<sub>14</sub></b> .....	98
<b>Figure 3.32</b> The normalized excitation and emission spectrum of <b>cBODIPY<sub>1</sub>-Pep<sub>14</sub></b> . .....	98
<b>Figure 3.33</b> Confocal imaging of <b>cBODIPY<sub>1</sub>-Pep<sub>14</sub></b> (green) and fluorescent α <sub>v</sub> β <sub>3</sub> -specific antibody (red) in T24, MRC5, and HeLa cell lines.....	99
<b>Figure 3.34</b> The fluorescent titration of <b>cDP<sub>1</sub>-Pep<sub>11</sub></b> with zinc(II) ion. ....	100

<b>Figure 3.35</b> Determination of stoichiometry between <b>cDP<sub>1</sub>-Pep<sub>11</sub></b> and zinc ion via Jobs' plot. The total concentration of <b>cDP<sub>1</sub>-Pep<sub>11</sub></b> and ZnCl <sub>2</sub> is 1 μM. ....	100
<b>Figure 3.36</b> The fluorescent responses of <b>cDP<sub>1</sub>-Pep<sub>11</sub></b> toward various metal ions. ....	101
<b>Figure 3.37</b> The zinc fluorescent titration for <b>cDP<sub>2</sub>-Pep<sub>11</sub></b> , <b>cDP<sub>4</sub>-Pep<sub>11</sub></b> , <b>cDP<sub>1</sub>-Pep<sub>12</sub></b> , <b>DP<sub>18</sub>-Pep<sub>18</sub></b> . ....	102
<b>Figure 3.38</b> MTT assay of <b>cDP<sub>1</sub>-Pep<sub>11</sub></b> toward (A) HeLa and (B) MRC5 cell lines. ....	103
<b>Figure 3.39</b> The changes in NMR spectrum of <b>cDP<sub>1</sub>-Pep<sub>11</sub></b> upon the addition of Zn(OAc) <sub>2</sub> . ....	104
<b>Figure 3.40</b> Confocal imaging of <b>cDP<sub>1</sub>-Pep<sub>11</sub></b> in HeLa and MRC5 cell line, as well as in HeLa cell line with zinc chelator TPEN. ....	104
<b>Figure 4.1</b> The building blocks for unnatural amino acids used in projects. ....	115
<b>Figure 4.2</b> The crude products of intermediate steps for synthesizing <b>cDP<sub>9</sub>-Pep<sub>11</sub></b> were cleaved, precipitated and analyzed by HPLC (gradient A) and ESI-MS. ....	135
<b>Figure 4.3</b> The synthetic route for XRGDX and their dipyrroin-cyclopeptide with Fmoc-X(Mtt)-OH (X = Lys, Orn, Dap). ....	136
<b>Figure 4.4</b> The synthetic route for XRGDX and their dipyrroin-cyclopeptide with Fmoc-X(Mtt)-OH (X = Dab). ....	140
<b>Figure 4.5</b> The crude products of intermediate steps for synthesizing <b>bcDP-Pep<sub>17</sub></b> were cleaved, precipitated and analyzed by HPLC (gradient A) and ESI-MS. ....	143