공학석사학위논문

Efficient Synthesis of Peptide Conjugated Perylene Diimide Bola-Amphiphile using 2-Chlorotrityl Chloride Resin and its Application to Photodynamic Therapy

2-CTC 수지를 이용한 효과적인 펩타이드-퍼릴렌의 합성 및 광역학치료로의 응용

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이 논문을 공학석사학위논문으로 제출함.

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ABSTRACT

Efficient Synthesis of Peptide Conjugated Perylene Diimide Bola-Amphiphile using 2-Chlorotrityl Chloride Resin and its Application to Photodynamic Therapy

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Perylene-3,4,9,10-tetra carboxylic diimide (PDI) has remarkable features as a photosensitizer in Photodynamic Therapy. However, PDI's poor solubility in aqueous condition limits its use. So it is commonly functionalized by various substituents on imide positions where two nitrogen groups comprised with imide moiety. However, there has been a technical limitation to conjugate them with proper amine gorups such as amino acids or peptides having pi-electron system. To overcome this problem, solid-phase peptide synthesis (SPPS) method has been utilized to conjugate peptides having diverse pi-electron functional molecules.

But still other problems appeared such as low yield with harsh reaction condition. Therefore, a new method was designed to overcome such disadvantages of conventional SPPS method. For this, reaction times, loading level, and solvent system were optimized. In this research, RGD sequence was conjugated as a substituent on imide position. The RGD sequence is well known as a cell attachment component. Thus, we could develope very useful materials which has both strong cell adhesion ability and photosensitizer for photodynamic therapy.

Keywords**:** perylene-3,4,9,10-tetracarboxylic dianhydride, solid phase peptide synthesis, RGD sequence, photodynamic therapy

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LIST OF ABBREVIATIONS

AcOH	Acetic acid
BOP	Benzotriazol-1-yloxy-tris(dimethylamino) Phosphonium hexafluorophsphate
BSA	Bovine serum albumin
DCM	Dichloromethane
DIC	Diisopropylcarbodiimide
DI water	Deionized water
DIPEA	*N*,*N*-Diisopropylethylamine
DMF	*N*,*N*-Dimethylformaide
EA	Ethyl acetate
ESI-MS	Electrospray ionization-mass spectrometry
EtOH	Ethanol
FITC	Fluorescein isothiocyanate
Fmoc	9-Fluorenylmethyloxycarbonyl
Fmoc-TEG-SA	*N*-1-(9-Fluorenylmethoxycarbonyl)-1,13-diamino-4,7,10-trioxatridecan-succinamic acid
FT	Fourier transform
HATU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HOBt	*N*-Hydroxybenzotriazole
HPLC	High-performance liquid chromatography
KCN	Potassium cyanide
MeCN	Methyl cyanide
MeOH	Methanol
MS	Mass spectrometry
NHS	*N*-hydroxysuccinimide
NMR	Nuclear magnetic resonance
PBS	Phosphate buffered saline
PBST	0.05 % TWEEN 20 in PBS buffer

Rbf	Round-bottom flask
SA	Succinic anhydride
SBP	Streptavidin binding peptide
SPPS	Solid-phase peptide synthesis
TFA	Trifluoroacetic acid
THF	Tetrahyrdofuran
TIPS	Triisopropylsilane
TWEEN 20	Polyoxyethylene-20-sorbitan monolaurate
UV	Ultraviolet
Vis	Visible

1. Introduction

1. 1 Perylene-3,4,9,10-tetra carboxylic diimide

Perylene-3,4,9,10-tetra carboxylic diimide (PDI) has remarkable features such as near-unity fluorescence quantum yields, high photochemical stability, and strong electron-accepting character.[1-3] Because of these unique features, PDI has attracted much attention in various fields such as organic photovoltaics, organic field-effect transistors, bio-labeling, sensors, single molecular spectroscopy, and supramolecular assemblies.[4]

However, PDI's poor solubility in aqueous condition limits its use in diverse fields. So it is commonly functionalized by various substituents on imide positions where two nitrogen groups comprised with imide moiety (Figure 1).[5-6] Substitutions at the imide position does not disrupting the planar nature of the aromatic core. In fact, there have been many studies which provided enhancing PDI's chemical and physical properties via various substituents.[7]

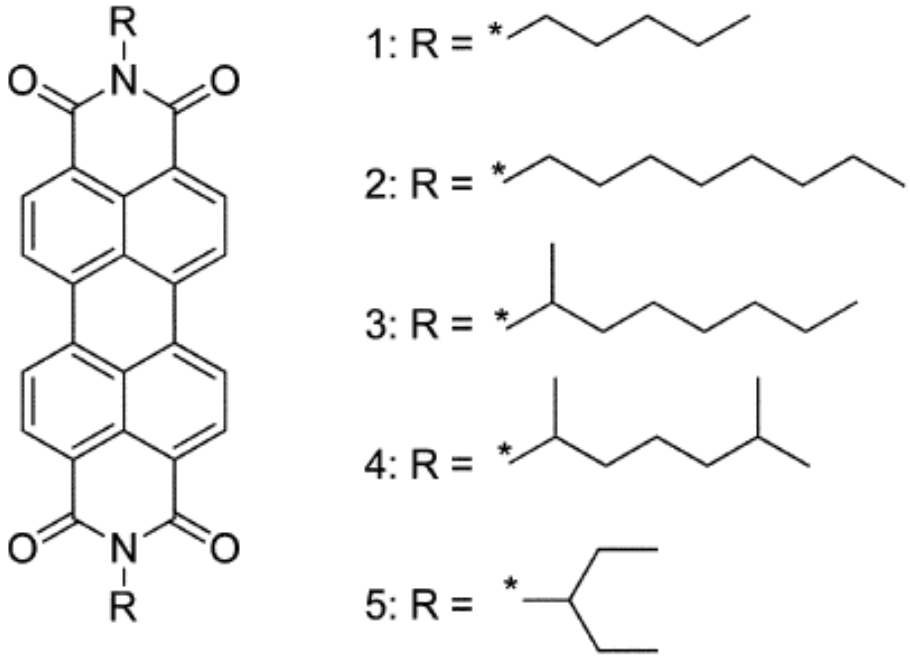


Fig 1. Various alkyl chains for introduced at the PDI imide position.

1. 2 Peptide-conjugated pi-electron material

In bionanotechnology, supramolecular biomaterials with pi-electron functionality have shown high performance.[4, 6, 8] Inspired by the self-assembly in natural system, synthetic peptides have drawn great interest as a powerful tool of molecular self-assembly agents.[8-10] Certain peptide amphiphiles have shown to exhibit sequence dependent self-assembly into well-defined structures such as, fibers, sheets, vesicles, micelles, and sacs (Figure 2).[11] Theses self-assembled nanostructures have been explored for use as molecular encapsulation and delivery assembles, mineralization templates, magnetic resonance imaging contrast agents, and supported networks for regenerative medicine.[12-15]

To obtain such functional materials, peptides were introduced by condensing with pi-electron material in molten imidazole.[13] However, there is a technical limitation to conjugate proper peptide sequences with pi-electron material.[14-15] For example, both the peptide amino terminus and the amine groups of side chain such as Lys can be involved in the condensation reaction, which produces undesired side products.[16] To overcome this problem, solid-phase peptide synthesis (SPPS) method was utilized to conjugate diverse pi-electron functional

molecules with peptide (Figure 3). However, there are still other problems such as low yield with harsh reaction condition. Therefore, a new method was designed to overcome such disadvantages of conventional SPPS method.

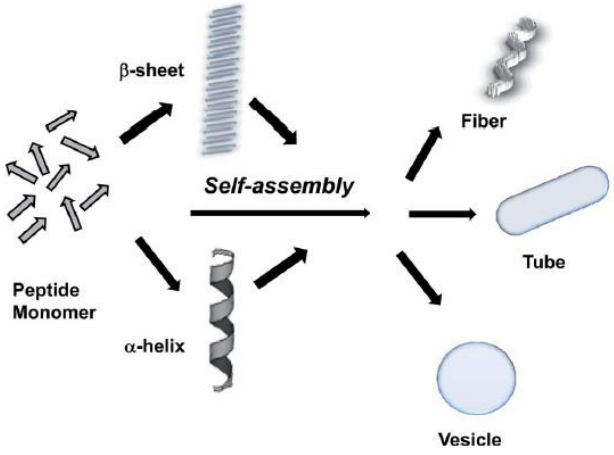


Fig 2. Self-assembly of peptides into different shapes of nanostructures.

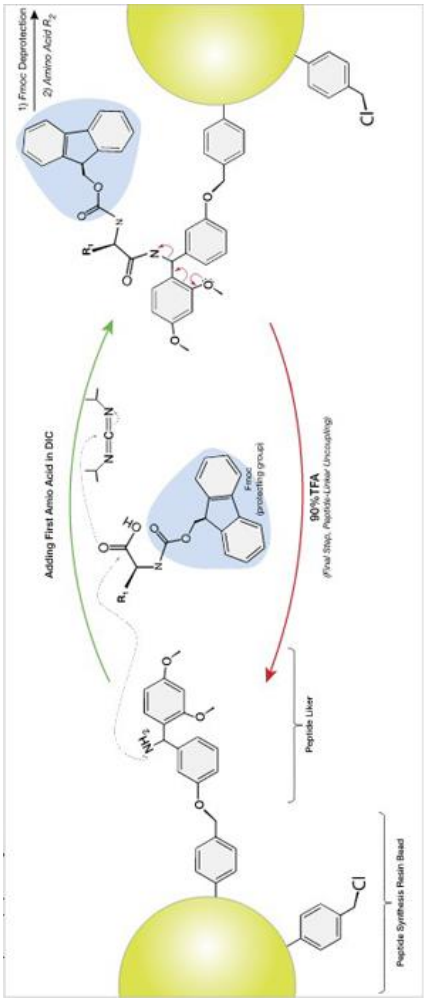


Fig 3. Solid-phase peptide synthesis on Rink amide resin, using Fmoc-protecte amino acids.

1. 3 Peptide-conjugated PDI for photodynamic therapy

Photodynamic therapy (PDT) is an efficient treatment for various types of cancer. Living cell's activities are easily suppressed by treating a photoactive molecule with molecular oxygen in the presence of light. Compared to other radiation therapy, PDT is nontoxic on the biological system.[11-12, 15-17] Various kinds of photosensitizers were used in PDT, such as porphyrin, chlorins, bacteriochlorins, phthalocyanines, metallophthalocyanines, porphycenes, squaraines, cyanines. Some of these photosensitizers have been used in clinical field for cancer treatment.[18-19]

PDI has many advantages in bioimaging and gene delivery because of high molar absorptivity, prominent chemical, thermal and photochemical stability. Also it has been utilized as a photosensitizer for generation of singlet oxygen (Figure 4).[7, 10-11, 13]

To use PDIs for PDT, a tripeptide sequence, Arg-Gly-Asp (RGD), was introduced to PDI as a peptide ligand. The RGD sequence is well known as a cell attachment site in a number of adhesive extracellular matrix, and cell surface proteins, and nearly half of over 20 known integrins recognize this sequence as their adhesion ligands (Figure 5).[7-8,

[13] These features of RGD sequence were combined with PDI for PDT.

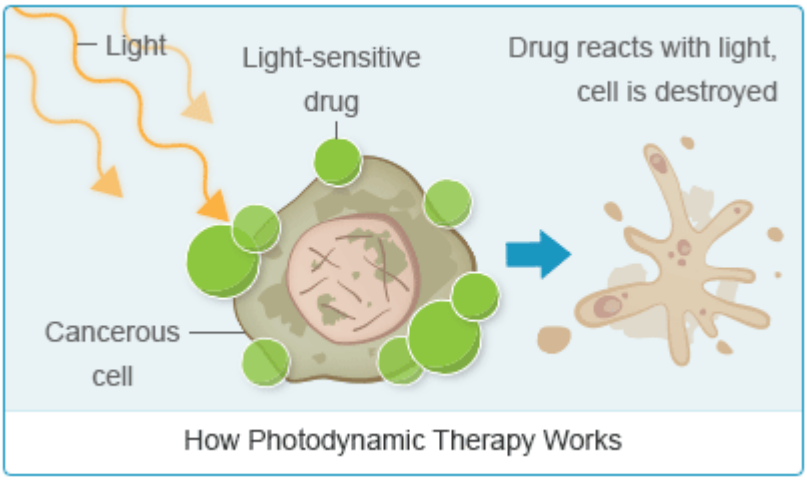


Fig 4. Principle of Photodynamic Therapy.

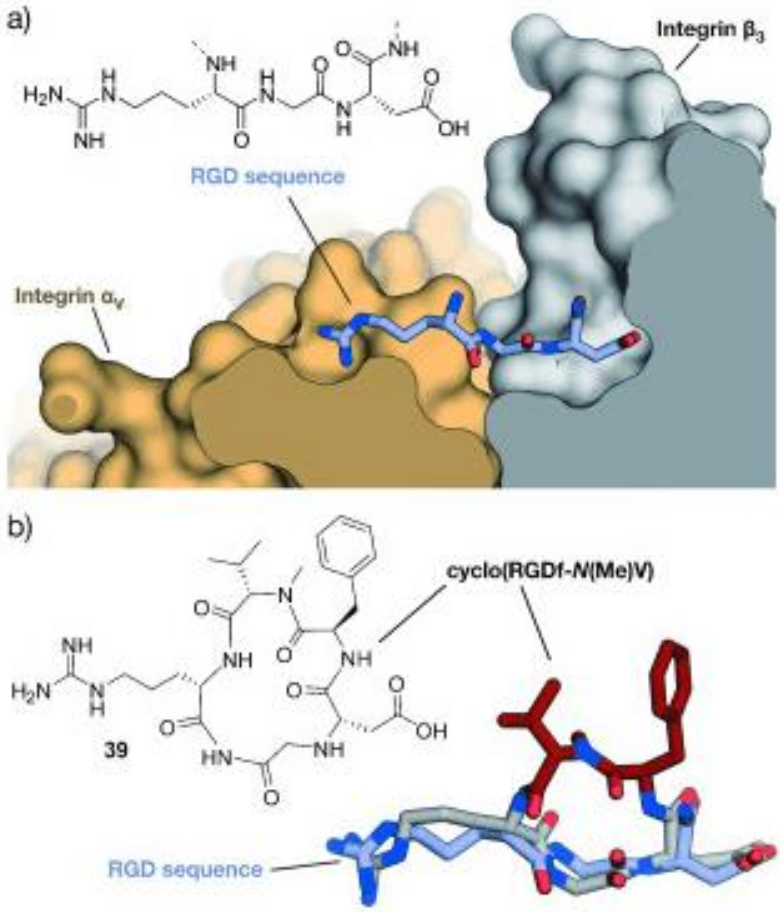


Fig 5. Interactions between RGD and integrin.

1. 4 Research Objective

In this thesis, we developed new method to overcome such as low yield with harsh reaction condition while conjugating peptide with perylene by SPPS. New method was optimized with time, loading level and solvents. RGD sequence was conjugated as substituents on imide position. And RGD sequence is well known as a cell attachment. For this reason, we could make useful material which has both strong cell adhesion ability and photosensitizer for photodynamic therapy.

2. Experimental Section

2. 1 General

Materials

2-Chlorotrityl resin (100-200 mesh, 1.04 mmol/g), 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU), 1-hydroxybenzotriazole (HOBt) anhydrous and filtered reactors (Libra tube) were purchased from BeadTech Inc. (Korea). Perylene-3,4,9,10-tetracarboxylic dianhydride from TCI (Japan), Ninhydrin, triisopropylsilane (TIPS), bovine serum albumin (BSA) and sodium cyanoborohydride were purchased from Sigma-Aldrich Co. (USA). Phenol was purchased from Junsei Chemical Co. Ltd. (Japan). Fmoc-Asp(otBu)-OH, Fmoc-Glycine-OH, Fmoc-Arg(pbf)-OH was purchased from BeadTech Inc. (Korea). Benzene, magnesium sulfate ($MgSO_4$) anhydrous, hydrochloric acid (HCl), trifluoroacetic acid (TFA), tetrahydrofuran (THF), ethyl acetate (EA), methyl cyanide (MeCN), dichloromethane (DCM), *N*,*N*-dimethylformamide (DMF), methanol (MeOH), ethanol (EtOH), acetic anhydride, *N*,*N*-diisopropylethylamine (DIPEA), piperidine, pyridine

and thionyl chloride were purchased from Daejung Chemicals & Metals Co. (Korea). Potassium cyanide (KCN) was purchased from DC Chemical Co. Ltd. (China). SPHERO^{TM} Streptavidin coated fluorescent yellow particles (0.44 um, 0.1 % w/v) was purchased from Spherotech Inc. (USA).

Instruments

UV absorbance was measured by using Optizen 2120 UV (Mechasys, Korea). Fluorescent images were taken by using LSM 780 confocal laser scanning microscope (Carl zeiss, Germany). Libra tubes were shaken by using SI-600R shaker (Jeio tech., Korea). Purity of synthesized peptide was analyzed by using LCQ LC/MS system (Thermo Finnigan, USA).

2. 2 Synthesis of L-alanine conjugated perylene dimide (PDI-$[A]_2$)

To synthesize PDI-$[A]_2$ L-alanine (180 mg, 2 mmol) and perylene-3,4,9,10-tetracarboxylic dianhydride (392 mg, 1 mmol) were mixed in 2 g of imidazole. The mixed product was stirred for 30 min at 130 ℃ under nitrogen atmosphere. After 30 min, the temperature was lowered to 90 ℃ and 5ml of DW was added. After 10 min, the product was filtered to remove unreacted product.. Then, 1M of HCl (10 mL) was added to convert the ionized product into free acid form. Final product was centrifuged and washed with DW several times until the washings is not acidic. It was dried in vacuum oven for 1 day.

2. 3 Synthesis of PDI-[ARGD]$_2$ by SPPS method

2-CTC resin (1g, 1.04 mmol/g) was added into each of three fritted PE tubes to make different kinds of loading level. Fmoc-Asp(otBu)-OH (0.3, 0.6, 0.9 mmol) each and DIPEA (3 eq.) in 10ml (HPLC)-DMF was introduced to the 2-CTC resin containing fritted PE tubes. The reaction was proceeded for overnight. After the reaction, the remaining amino groups were capped by treating with 10ml MeOH and DIPEA (3 eq.) for 3 hr. The Loading level was calculated by Fmoc titration. To conjugate the next amino acid, 10ml of 20% piperidine/DMF treated 20 min that Fmoc groups were removed from the loaded resin. The rest amino acid was coupled with based on the loading level, HOBT (2 eq.) HBTU (2 eq.) Fmoc-Glycine-OH (2 eq.) and DIPEA (3 eq.) in 10ml DMF. For 3 hr in a shaking incubater, the resin was washed with DMF, DCM, MeOH 3 times each and Fmoc-Arg(pbf)-OH was conjugated by the same path. The resulting Fmoc-Arg(pbf)-Gly-Asp(tBu)-CTC resin was treated with 20% piperidine/DMF for 20 min and dried. Then, the resins were divided into four groups, 200mg each, to search for the optimal reaction times and loading levels. The deprotected H-Arg(pbf)-Gly-Asp(tBu)-CTC resins were coupled to PDI-[A]$_2$ with HOBT (1 eq.),

HBTU (1 eq.), DIPEA (1 eq.) and A-PDI-A (0.5 eq.) in DMF for 30 min, 1 hr, 2 hr, 4 hr, 12 hr, respectively. The resin was washed with DMF, DCM, MeOH until there was no fluorescence from the washing solution. For on-bead dimerization, the same coupling condition, HOBT (1 eq.), HBTU (1 eq.), and DIPEA (1 eq.) in DMF, was applied. The dimerization time was same as previous reaction time. Final product was cleaved from the resin by treating resin with TFA/TIPS/H_2O (95:2.5:2.5) solution for 1 hr and recovered as red solids by ether precipitation. The yield of product was compared with HPLC and ESI-MS. To find out the swelling effect of resin on the synthesis, different kinds of solvents were also compared. DMF and two mixed solvents (THF/DMF, DCM/DMF) were used for SPPS. The loading level and the coupling reaction time were fixed 0.9 mmol/g and 2 hr. These results were also compared with HPLC results.

2. 4 Reverse phase silica gel column chromatography

After confirmed the structure of each peak by HPLC and ESI-MS, reverse phase silica gel column chromatography was used to obtain the final product. Fully end-capped C8-reversed phase silica gel was prepared and the reverse phase tlc plate was also used to get reasonable Rf value for separation. Under the 40:60 MeCN : H_2O w/ 0.1% TFA solvent system, we could get 0.44 Rf value of product and 0.75 Rf value of by-product which peptide only conjugated one side of PDI. First of all, silica was flushed with distilled MeOH to pack. After loading sample on the sea sand, mobile solvent system was added for separation. higher pressure was generated than normal-phase flash chromatography and could see two different products was separated while coming down. The product earned by column chromatography also confirmed by ESI-MS.

2. 5 Cell preparation

3T3 cell line was obtained from Sigma-aldrich. 3T3 cell line was grown in RPMI-1640 complete medium. The complete media for 3T3 cell line was supplemented with 10% fetal bovine serum and 1% penicillin/streptomycine (Sigma-Aldrich). The cells were incubated at 37 ℃ in a humidified atmosphere containing 5% CO_2. The cells were seeded into 96-well plate. They were incubated overnight at 37 ℃ in a humidified atmosphere containing 5% CO_2. Next day, they were washed with sterile PBS and incubated 2 hr with media which have only 1% penicillin/streptomycine at 37 ℃ in a containing 5% CO_2. After 2 hr, they were washed with sterile PBS. Each cells were treated 0.1mM, 0.01mM, 0.001mM, 0.0001mM of PDI-$[ARGD]_2$ in 1% penicillin/streptomycien media overnight.

2. 6 Cell viability test

3T3 cells were seeded (1×10^4 cells/well) onto 96-well plates, and incubated for 24hr. After replacing the culture medium with 200 μL of serum-free medium containing 0.1 mM, 0.01 mM, 0.001 mM, 0.0001 mM of PDI-$[ARGD]_2$, the treated cells were incubated for 2 hr at 37 ℃. The cells were washed with fresh medium and each well was treated with 20 μL of CCK-8 reagent and incubated for 2 hr. And then absorbance was measured at 450 nm using SpectraMax M2.

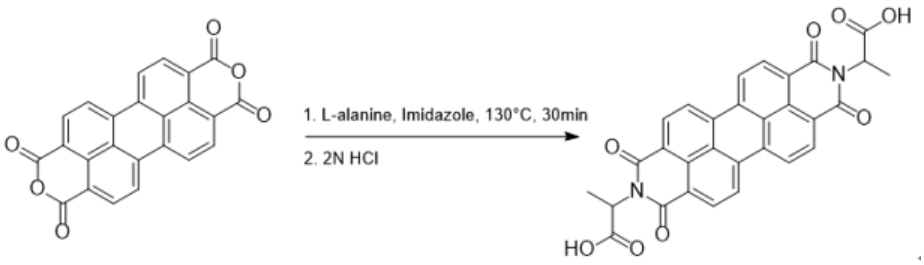
2. 7 Photodynamic therapy using PDI-$[ARGD]_2$ and PDI-$[A]_2$

3T3 cells were seeded (1 x 10^4 cells/well) on to 96-well plates, and incubated 24 hr. After replacing the culture medium with 200 uL of serum-free medium containing PDI-$[ARGD]_2$ or PDI-$[A]_2$ (each 1mmol), the treated cells were incubated for 2 hr at 37 ℃. The cells were washed with fresh medium and irradiated with laser (535 nm, 200nW/cm^2, each well for 5 min). And then the cells were further incubated for another 3 hr to determine the phototoxicity. For dark-toxicity, the cells were prepared following the same procedure without laser irradiation. At last, each well was treated with 20 uL of CCK-8 reagent and incubated for 1 hr, and then absorbance was measured at 450 nm using SpectraMaX M2 (Molecular Devices, USA)

3. Results and discussion

3. 1 Synthesis of PDI-$[A]_2$

Scheme 1 shows how to synthesize A-PDI-A. First, 1 mmol of perylene-3,4,9,10-tetracarboxylic dianhydride and 2 mmol of L-alanine was stirred in molten imidazole at 130 ℃ for 30 minutes. Before finishing the reaction, the temperature was lowered to 90 ℃ and 5ml of DW was added. After 10 min of stirring, the product was filtered to remove unreacted product. And then 1M of HCl was treated to ionized product and washed with DW several times. Product was collected with filter glass and dried in vacuum oven 1 day. It was confirmed with NMR and ESI-mass (appendix).

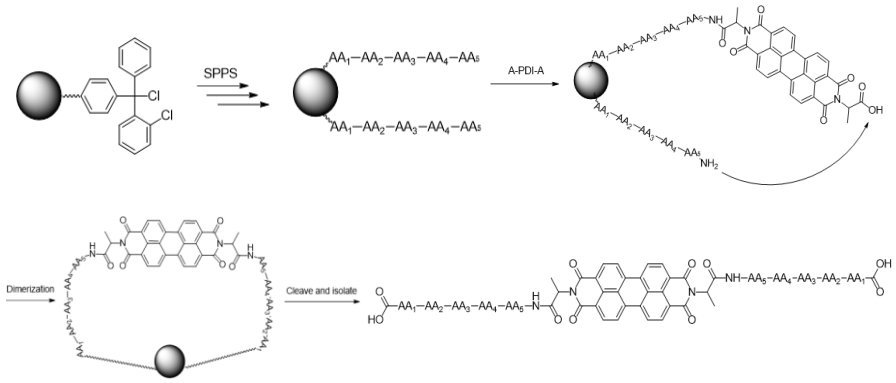


Scheme 1. Synthesis A-PDI-A in molten imidazole.

3. 2 Synthesis and characterization of PDI-$[ARGD]_2$

RGD sequence was coupled to PDI-$[A]_2$ by SPPS method to afford PDI-$[ARGD]_2$ (Scheme 2). Three different quantity of Fmoc-Asp(tBu)-OH were coupled to CTC resin in DMF for overnight. After the loading level was calculated by Fmoc titration and Fmoc-Gly-OH, Fmoc-Arg-(pbf)-OH were coupled to the Asp-CTC resin, sequentially. After all sequence was coupled to the resin, the time was optimized. Each of them were reacted with PDI-$[A]_2$ 30 min, 1 hr, 2 hr, 4 hr, 12 hr and dimerization was also proceeded at the same time (scheme 2). After reaction is finished, the color of resin formed dark-red with strong fluorescence under 365 nm (Figure 6a). The product cleaved from the resin also shows strong fluorescence of the same wavelength (Figure 6b). Yield of product were compared using HPLC. The peak of monopeptide-PDI which is more hydrophobic than the dipeptide-PDI, around 15.2 min and that of dimerized product around 9.8 min, confirmed by ESI-MS (appendix). The yield of the product obtained by the integration of HPLC peaks is summarized (Figure 7). Increasing reaction time and high loading of peptide induced higher product yield. As expected, dimerization was affected by the density of the peptide

groups on CTC resin. Longer reaction time and higher loading gave good result of dimerized peptide-PDI conjugate resin. Various solvent system which can affect the swelling property of CTC resin were also compared (Table 1). DMF and two other mixed solvent system (THF/DMF, DCM/DMF) were used for SPPS. The loading level and reaction time were fixed 0.9 mmol/g and 2 hr, respectively. However, there was little difference of product yield with different kind of solvent system. It was expected that swelling property is direct factor for efficient synthesis of P-PDI bola-amphiphile due to controlling reaction site of the resin, but it was not critical factor in this case.



Scheme 2. Synthesis of peptide conjugated PDI by SPPS.

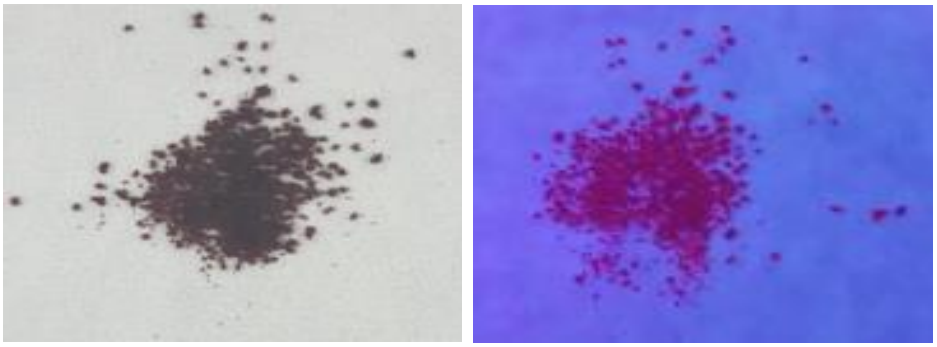


Fig 6a. Peptide conjugated PDI coupled resin showing strong fluorescence under 365nm.

Fig 6b. Peptide conjugated-PDI cleaved from CTC resin, showing strong fluorescence under 365nm.

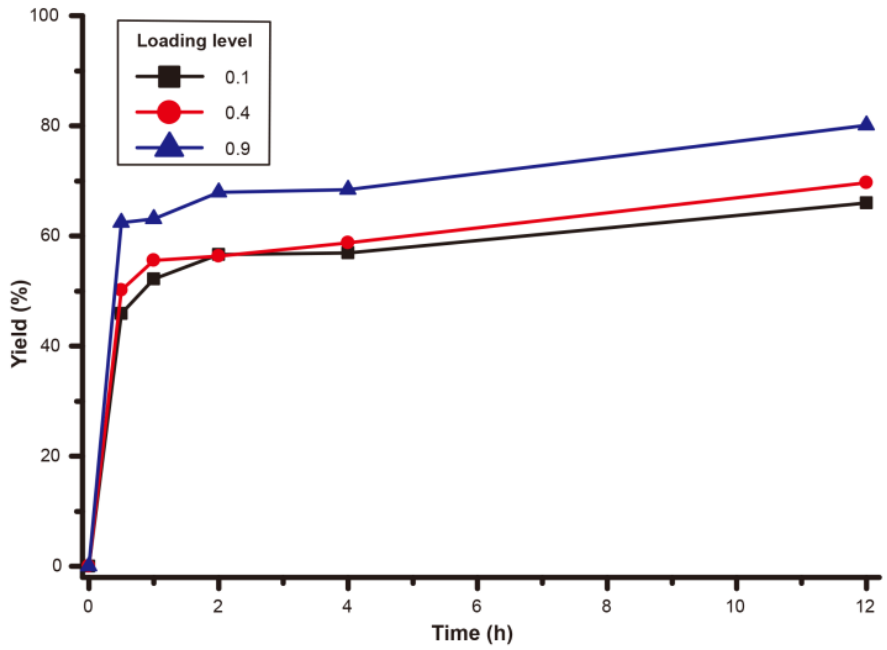


Fig 7. Coupling and dimerization yield vs reaction time depending on loading level of CTC resin (black: 0.1 mmol/g, red: 0.4 mmol/g, blue: 0.9 mmol/g)

Table 1 Swelling Property of CTC Resin and Dimerization Yield of P-PDI in Various Solvents

Solvent	Swelling volume(mL/g)	Yield (%)
DMF	2	49.5
DMF/THF	2.2	53.1
DMF/DMC	2.4	57.1

3. 3 Optical properties of PDI-[ARGD]$_2$

UV-Vis spectra of PDI-[ARGD]$_2$ (Figure 8a) showed that three distinct absorption bands were emerged at 532, 490, and 460 nm in DMF-H_2O mixture solvent. The highest observance was obtained at 532 nm. Upon addition of water, the absorption peak shifted to long wavelength and absorbance was decreased. This result suggest that PDI-[ARGD]$_2$ could be used as photosensitizer in Photodynamic Therapy. Fluorescence spectra also showed similar tendency as UV-vis spectra (Figure 8b). When the ratio of water goes up in the DMF-H_2O mixed solvent, intensity was decreased and fluorescence peaks were shifted long wavelength.

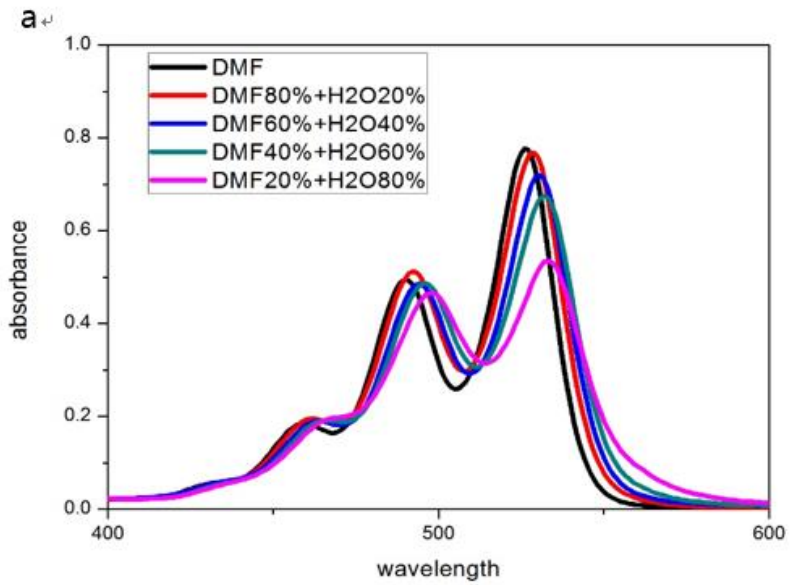


Fig 8a. UV spectra of PDI-[ARGD]$_2$ in DMF-H_2O mixture of different ratios.

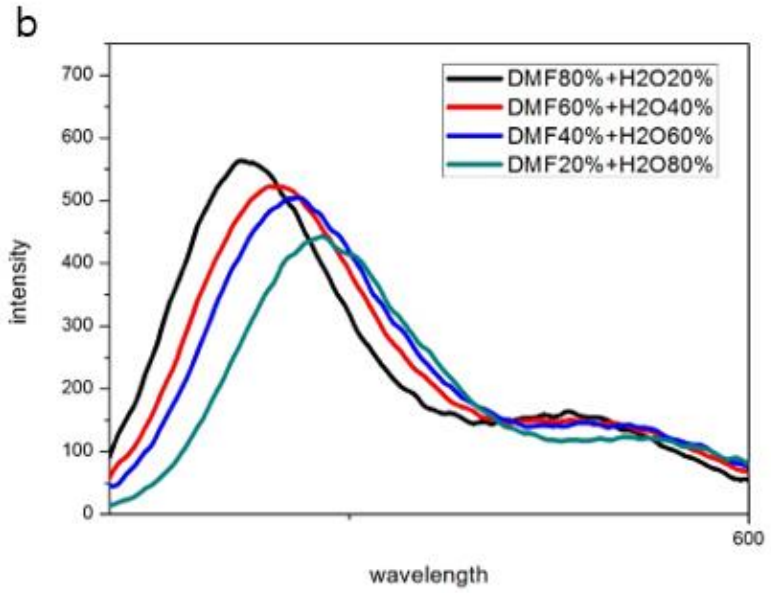


Fig 8b. Fluorescence spectra of PDI-[ARGD]$_2$ in DMF-H_2O mixture of different ratios.

3. 4 Cell toxicity test of PDI-$[ARGD]_2$ on 3T3 cells

3T3 cells were treated with PDI-$[ARGD]_2$ (0.1 mM, 0.01 mM, 0.001 mM, and 0.0001 mM) and incubated for 24 hr. After replacing the culture medium with fresh medium, each well was treated with 20 μL of Cell Counting Kit-8 (CCK-8) reagent, and incubated for 2 hr. Then the absorbance was measured at 450 nm to check cell viability (Figure 9). CCK-8 allows sensitive colorimetric assays for the determination of cell viability in cell proliferation and cytotoxicity assays. The principle of CCK-8 depicted in Figure 10.

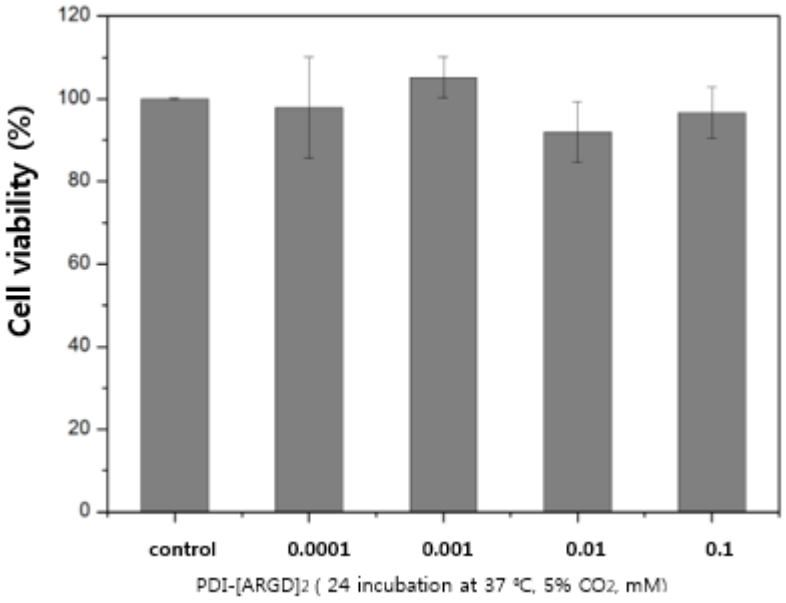


Fig 9. Cell viability result after treating with peptide conjugated-PDI.

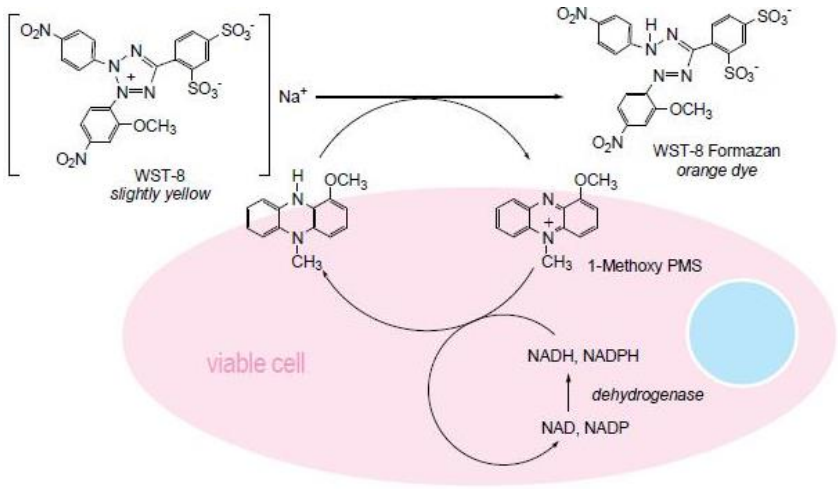


Fig 10. Principle of cell viability test with CCK-8.

3. 5 Fluorescence image of 3T3 cell line

To check the role of RGD sequence, PDI-$[ARGD]_2$ was treated to 3T3 cell lines. PDI-$[A]_2$ was also treated to the 3T3 cell line as a control to compare the differences between the two. The 3T3 cells were incubated with both of the two fluorescence peptide conjugated materials were for overnight. After fixing the cells with formaldehyde, DAPI was used for dying the cell nucleus. The fluorescence image was obtained with confocal microscope (Figure 11). As can be seen from Fig 9a, PDI-$[ARGD]_2$ showed strong fluorescence under 488 nm. On the other hand, PDI-$[A]_2$ rarely responded with 488 nm and only showed DAPI fluorescence at nucleus. Confocal image of the cell showed that the fluorescence signal appeared from the inside of the cell. This result confirmed that PDI-$[ARGD]_2$ was transported into the cell by endocytosis.

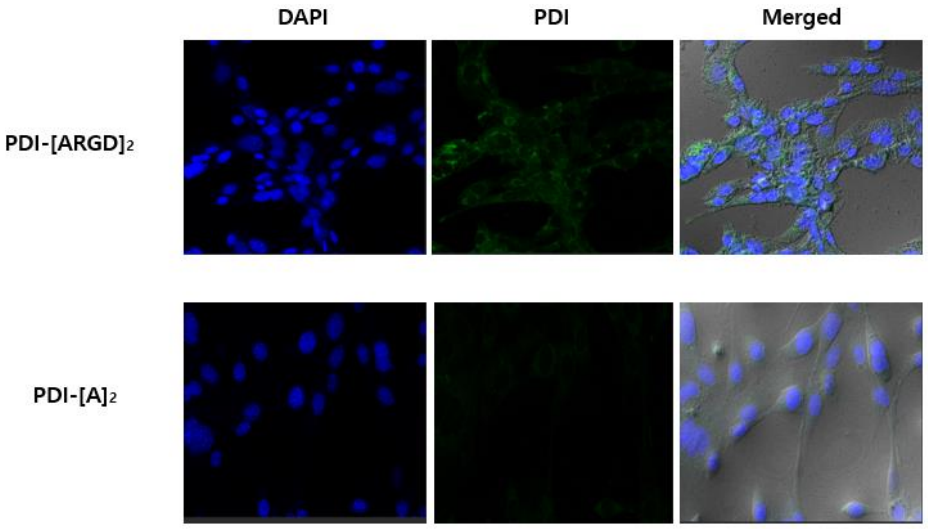


Fig 11. The fluorescence image of cells shows PDI-$[ARGD]_2$ was well transported into the cell by endocytosis. This result indicate RGD sequence is critical factor for photodynamic Therapy**.**

3. 6 Photodynamic therapy with PDI-$[ARGD]_2$

3T3 cells were seeded and incubated for 24 hr. After replacing the culture medium with 200 uL of serum-free medium containing PDI-$[ARGD]_2$ or PDI-$[A]_2$ (each 1mM), the treated cells were incubated for 2 hr at 37 ℃. The cells were washed with fresh medium and irradiated with laser (535 nm, 200nW/cm^2, each well for 5min). And then the cells were further incubated for another 3 hr to determine the phototoxicity. For dark-toxicity, the cells were prepared following the same procedure without laser irradiation. In dark-toxicity test, almost all the cells were survived in various concentration of PDI-$[ARGD]_2$ and PDI-$[A]_2$. After irradiating 535nm laser each, the cell viability after treating PDI-$[A]_2$ decreased but maintained as 85%. PDI-$[ARGD]_2$ shows outstanding result that the cell viability was dramatically decreased to 45%. These results indicate that PDI-$[ARGD]_2$ could be used in Photodynamic Therapy practically (Figure 12).

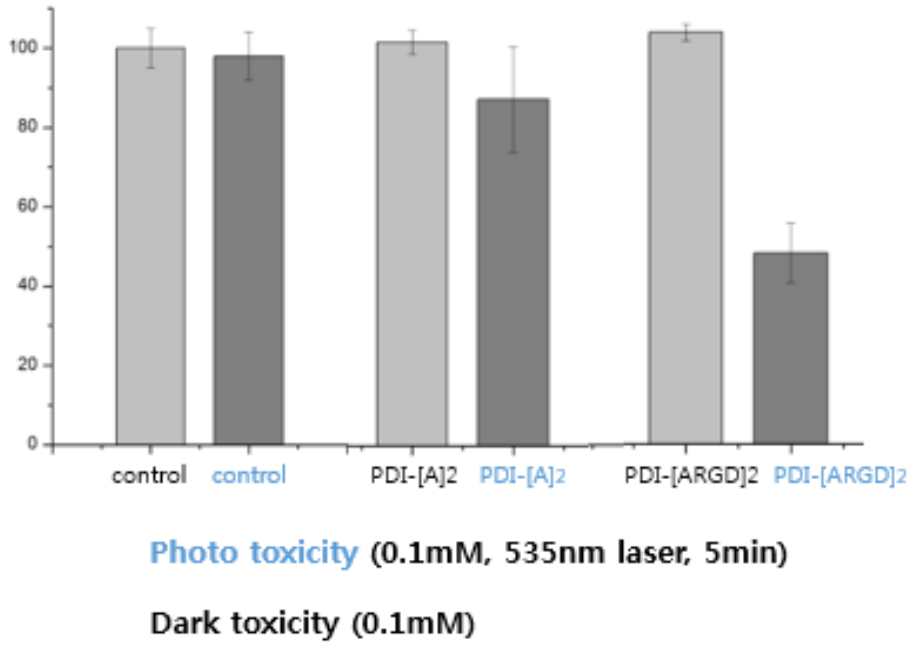


Figure 12. Almost cells were survived in non-light condition. However, after treating 535 nm laser to the cells for 5min, the cell viability of PDI-$[A]_2$ was decreased 85% and PDI-$[ARGD]_2$ was decreased 45%.

4. Conclusion

We designed a new method which can provide peptide conjugated perylene diimide (P-PDI) bola-amphiphile in more efficient way. By introducing a spacer amino acid, L-Ala, to perylene diimide (PDI) in molten imidazole, the solubility of perylene in organic solution was improved. Thus, the rest of the amino acid sequences can be conjugated to PDI-$[A]_2$ via solid phase peptide synthesis method. A model peptide sequence, Arg-Gly-Asp (RGD), which can mediate cell attachment, was chosen for cell target therapy. We found that reaction time and loading level were critical factors for the synthesis of P-PDI bola-amphiphile on CTC resin. Possibility of photodynamic therapy with PDI-$[ARGD]_2$ was also tested on cells, and gave a good performance, which suggest that PDI-$[ARGD]_2$ has a great potential in cancer treatment.

5. References

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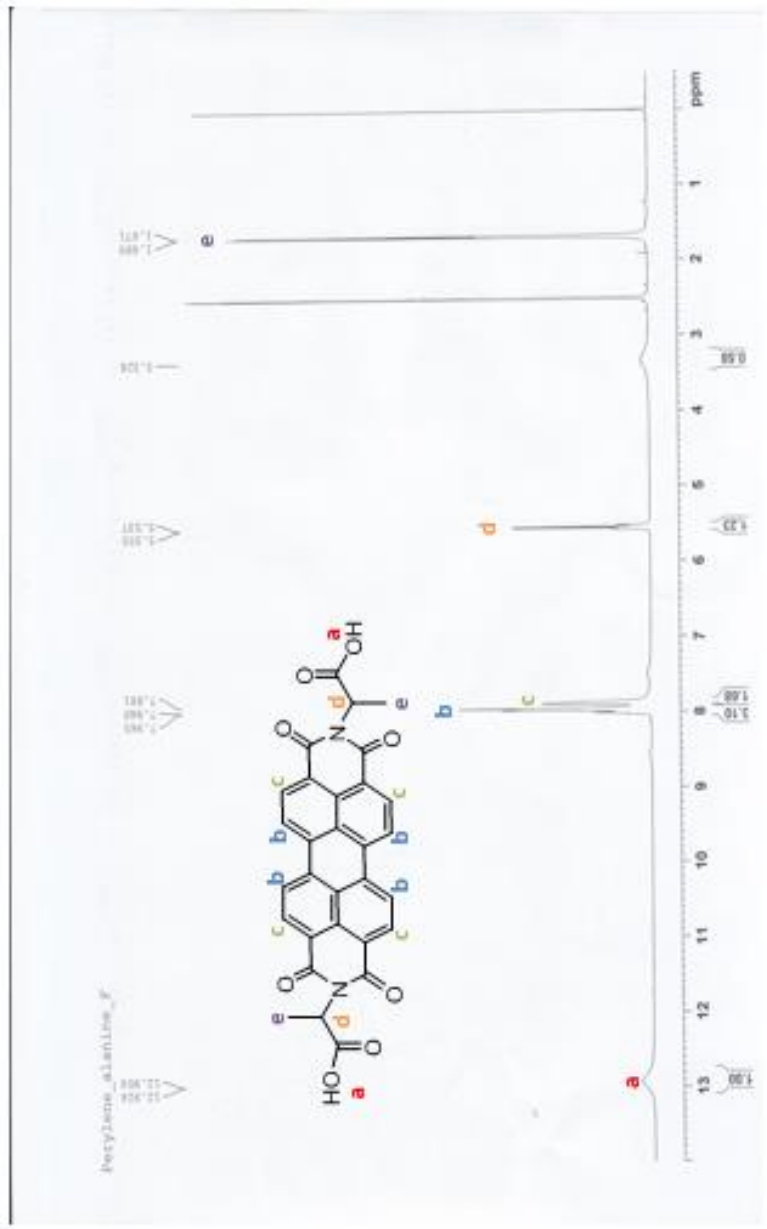
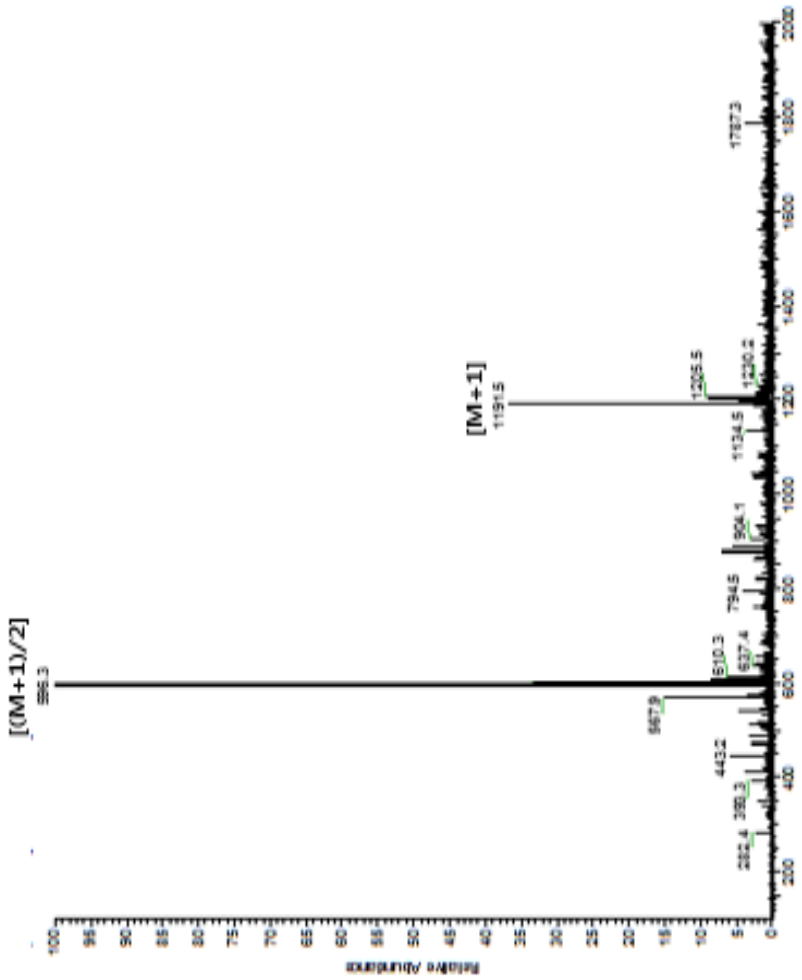
Appendix 1



Appendix 2



6. Abstract in Korea

요 약

퍼릴렌 테트라 카르복실릭 다이이미드는 다양한 특징을 지니고 있고 광감각제로 광역학치료에 쓰인다는 연구가 있다. 하지만 퍼릴렌 테트라 카르복실릭 다이안하이드라이드의 수용액 상에서의 낮은 용해성 때문에 사용이 제한적이므로 다이이미드 쪽에 치환기를 결합하여 실질적으로 활용되고 있다. 기존 용액상에서의 원하는 펩타이드 시퀀스와의 결합은 어려움이 있었고 이를 극복하기 위해 고체상 펩타이드 합성법에 의한 결합이 고안되었다. 하지만 보고된 퍼릴렌 테트라 카르복실릭 다이안하이드라이드와 고체상 합성법에 의한 펩타이드 결합은 낮은 수율 및 까다로운 반응 조건이 필요했다. 이를 극복하기 위해 새로운 방법이 고안되었고 높은 수율을 통해 만든 물질을 합성 할 수 있었다. 퍼릴렌 다이이미드에 아르기닌-글라이신-아스파틱 엑시드를 시퀀스를 결합시켜 세포의 부착 성질을 부여하였고 퍼릴렌을 광감각제로 이용하여 광역학 치료에 사용 될 수 있는 물질을 개발하였다.

주요어: 퍼릴렌 카르복실릭 다이이미드, 고체상 펩타이드 합성법, 광감각제, 광역학치료

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