Synthesis of Enediyne-Chlorophyll Derivatives as Cytotoxic Conjugates

Jin-Jun Wang,[a,*] Kee-In Lee,[†] Mun-Hwan Kim,[*] and Young Key Shim[†,‡,*]

[*]*Bio-Organic Science Division, Korea Research Institute of Chemical Technology, P.O. Box 107, Yusong, Daejeon 305-600, Korea*
[‡]*School of Nano Engineering, Inje University, Gimhae, Gyongnam 621-749, Korea*
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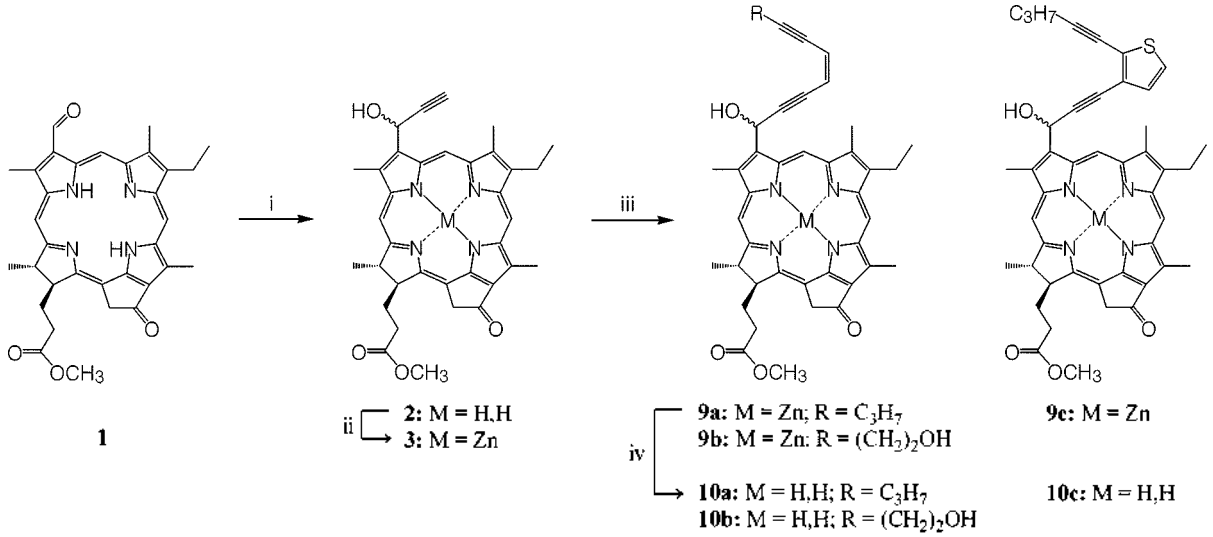
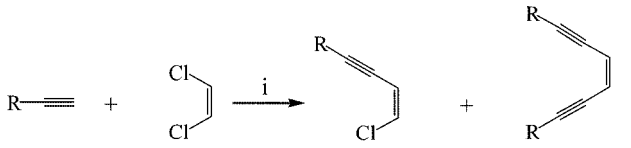
Conventional approaches to cancer treatment such as alkylating agents, platinum complexes, antimetabolites, and antitumor antibiotics are involved in cytotoxic intervention at the level of DNA replication. They act to block the biosynthesis or transcription of nucleic acids or to prevent cell division by interfering with mitotic spindles.[1] While cytotoxic drugs are most effective against rapid growing tumor cells, they have the undesired property of inhibiting the synthesis of DNA in normal cells. Thus, there still remains a critical need for the development of agents possessing selectivity against tumor cells over normal cells. In view of non-specific cytotoxicity of most chemotherapeutic agents against normal cells, an efficient targeting of chemotherapeutic drugs to the cancerous area could be of great benefit. One of the interesting approaches is a dyad conjugate targeting delivery of drug into the desired place *via* well-known ligand, which binds to receptors on the cells.[2] Most targeted cytotoxic conjugates, such as siderophore-β-lactam,[2a] LHRH-doxorubicin,[2b] somatostatin-paclitaxel,[2c] and porphyrin-platinum(II) complex[2d] are hybrid molecules composed of a carrier and a cytotoxic moiety.

A wide variety of porphyrin-based photosensitizers including porphyrins, chlorins, and bacteriochlorins have been used and suggested as photosensitizers in photodynamic therapy. The enigma of the selective accumulation of porphyrin into tumor site spurred exhaustive biomedical and photophysical studies.[3] Such interesting biochemical properties of porphyrins prompted us to develop a new generation of enedyine-porphyrin conjugates. Over the past few years, intense research has been concerned with a new class of antibiotics, the enediynes, which are some of the most potent antitumor agents ever discovered. They contain a *cis*-hex-3-ene-1,5-diyne unit embedded within a strained 10-membered ring, which is capable of undergoing a cycloaromatization process to generate a benzenoid diradical.[4] This diradical is capable of abstracting hydrogen atoms from the sugar part of DNA, which is responsible for DNA cleavage. Thus, we have interests in designing the structurally simplified enediyne unit hanging on porphyrin in a single entity. We reasoned that benefits of tumor-localizing porphyrin molecules are able to support selective tumorcidal action of enediynes. Here, we'd like to report the preliminary

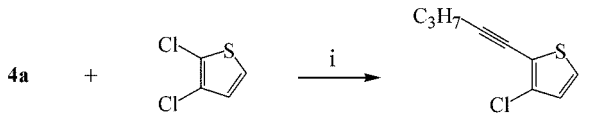


Scheme 1. *Reagents and conditions:* i, BrMgCCH, 45%; ii, $Zn(OAc)_2$, 98%; iii, **7a-c**, $Pd(PPh_3)_4$, PPh_3, CuI, *n*-$BuNH_2$, 62% (**9a**); 58% (**9b**); 60% (**9c**); iv, TFA, 58% (**10a**); 64% (**10b**); 67% (**10c**).

*Corresponding Author. Tel.: +82-55-320-3871; Fax: +82-55-320-3631; e-mail: ykshim@inje.ac.kr
[a]Current address: Department of Applied Chemistry, Yantai University, Yantai, Shangdong 264005, China



4a: R = C_3H_7 **5** **7a**: R = C_3H_7 **8a**: R = C_3H_7
4b: R = $(CH_2)_2OH$ **7b**: R = $(CH_2)_2OH$



6 **7c**

Scheme 2. *Reagents and conditions:* i, $Pd(PPh_3)_4$, PPh_3, CuI, *n*-$BuNH_2$, 58% (**7a**) and 21% (**8a**); 53% (**7b**); 60% (**7c**).

results on synthesis of enediyne-carrying chlorophyll derivatives as cytotoxic conjugates, as shown in Scheme 1.

The methyl pyropheophorbide-d (MPPd, **1**) was prepared from methyl pyropheophorbide-a (MPPa), which was extracted from the alga *Spihurina maxima*.[5] The isolated MPPa was then subjected to the oxidation with osmium tetroxide in the presence of the catalytic amount of pyridine, followed by the oxidative cleavage with sodium periodate to afford MPPd, according to the reported procedures.[6] Grignard reaction on 3-formyl group of **1** with ethynylmagnesium bromide gave the propargyl alcohol derivative **2** as diastereomeric mixtures, in 45% yield. Then, **2** was readily converted to the Zn-complex **3** to prevent from a plausible transmetalation with the catalyst metal ions in the next reaction conditions. Because more vigorous conditions are necessary for the demetalaton of copper porphyrins,[7] the metalation was performed with large excess of zinc acetate in methylene chloride to give **3** in a quantitative yield.

In general, the Pd/Cu-catalyzed coupling between sp- and sp^2-carbon centers is particularly valuable as a method for the construction of the 3-ene-1,5-diyne moiety. The required vinyl chlorides, **7a** and **7b**, were prepared from *cis*-1,2-dichloroethylene (**5**) with 1-pentyne (**4a**) and 3-butyn-1-ol (**4b**), respectively, in the presence of catalytic amounts of $Pd(PPh_3)_4$ and CuI in *n*-butylamine (Scheme 2). During the preparation of **7a**, the bis-coupled alkene **8a** was isolated in 21% yield. The eneyne fused with aromatic compound **7c** was also obtained from 2,3-dichlorothiophene (**6**) in moderate yield.

The final installation of enediyne was preformed with the terminal alkyne on the zinc-chlorin **3** with *Z*-chloroeneynes **7a-c** under the previously described Sonogashira coupling conditions. The removal of zinc in **9a-c** with TFA was smoothly proceeded to afford the enediyne-chlorophyll derivatives **10a-c**, respectively, as the inseparable diastereomeric mixtures.[8]

It is noteworthy that the enedyine-chlorin conjugates prepared in this study are jointed them in the single entity not by the aid of connecting spacers. It is expected that benefits of tumor-localizing porphyrin molecules are able to support selective tumorcidal action of enediynes. Further synthesis of different kinds of enediyne-porphyrin conjugates is still undergoing and photochemical and biochemical studies will be reported in due courses.

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References

1. Paull, K. D.; Hamel, E.; Malspeis, L. In *Cancer Chemotherapeutic Agents*; Foye, W. O., Ed.; American Chemical Society: Washington DC, 1995; p 9.
2. (a) Ghosh, A.; Ghosh, M.; Niu, C.; Malouin, F.; Moellmann, U.; Miller, M. J. *Chemistry & Biology* **1996**, *3*, 1011. (b) Schally, A. V.; Nagy, A. *Eur. J. Endocrinol.* **1999**, *141*, 1. (c) Huang, C.-M.; Wu, Y.-T.; Chen, S.-T. *Chemistry & Biology* **2000**, *7*, 453. (d) Song, R.; Kim, Y.-S.; Lee, C. O.; Sohn, Y. S. *Tetrahedron Lett.* **2003**, *44*, 1537.
3. (a) Sternberg, E. D.; Dolphin, D. *Tetrahedron* **1998**, *54*, 4151. (b) Hsi, R. A.; Rosenthal, D. I.; Glatstein, E. *Drugs* **1999**, *57*, 725. (c) Oehsner, M. *J. Photochem. Photobiol. B: Biol.* **1997**, *39*, 1. (d) Shim, Y. K.; Pandey, R. K.; Smith, K. M. *J. Porphyrins Phthalocyanines* **2000**, *4*, 185. (e) Lee, J.-C.; Kim, T.-Y.; Kang, S. H.; Shim, Y. K. *Bull. Korean Chem. Soc.* **2001**, *22*, 257. (f) Ostrowski, S.; Mikus, A.; Shim, Y. K.; Lee, J.-C.; Seo, E.-Y.; Lee, K.-I.; Olejnik, M. *Heterocycles* **2002**, *57*, 1615.
4. (a) Nicolaou, K. C.; Smith, A. L. *Acc. Chem. Res.* **1992**, *25*, 497. (b) Danishefsky, S. J.; Shair, M. D. *J. Org. Chem.* **1996**, *61*, 16. (c) Smith, A. L.; Nicolaou, K. C. *J. Med. Chem.* **1996**, *39*, 2103. (d) Sonogashira, K. In *Comprehensive Organic Synthesis*; Trost, B. M.; Fleming, I., Eds.; Pergamon Press: New York, 1991; Vol. 3, p 521.
5. Smith, K. M.; Goff, D. A.; Simpson, D. J. *J. Am. Chem. Soc.* **1985**, *107*, 4946.
6. (a) Parker, S. E.; Brinigar, W. S. *Bioinorg. Chem.* **1976**, *5*, 325. (b) Kenner, G. W.; Quirke, J. M.; Smith, K. M. *Tetrahedron* **1976**, *32*, 2753. (c) Kahl, S. B.; Schaeck, J. J.; Koo, M.-S. *J. Org. Chem.* **1997**, *62*, 1875.
7. Smith, K. M.; Graig, G. W. *J. Org. Chem.* **1983**, *48*, 4320.
8. Representative procedure for **10b**. To a solution of **3** (0.2 mmol) in benzene (10 mL) was added $Pd(PPh_3)_4$ (45 mg), *n*-butylamine (0.3 mmol), and **7b** (0.3 mmol) under nitrogen atmosphere. And then, CuI (925 mg) was introduced into the solution. After the reaction mixture was allowed to stir for 8 hr at room temperature, quenched with a saturated NH_4Cl solution. The mixture was extracted with Et_2O. The organic layer was washed with water and brine, and dried over anhydrous Na_2SO_4 and concentrated. The concentrated was purified by column chromatography (eluent: hexane/EtOAc = 3/1) to give **9b** in 58% yield. The Zn-chlorin **9b** was dissolved in CH_2Cl_2 (15 mL), and then treated with 30% aq. TFA (15 mL). The solution was allowed to stir for 4 hr at room temperature. The organic layer was washed with water and 5% $NaHCO_3$ solution, and then dried over Na_2SO_4 and concentrated. The concentrated was purified by column chromatography (eluent: hexane/EtOAc = 3/1) to afford **10b** in 64% yield. UV/Vis ($CHCl_3$) λ_{max} (rel absorbance) nm 317 (0.38), 410 (1.82), 473 (0.10), 506 (0.22), 537 (0.21), 608 (0.18), 665 (0.98); ^1H NMR ($CDCl_3$) δ 9.80, 9.27, 8.52 (s, each 1H), 7.07 (m, 1H), 5.68 (brs, 2H), 5.14-4.95 (m, 2H), 3.62 (q, 2H, J = 7.2 Hz), 3.60, 3.45, 3.44, 3.20 (s, each 3H), 2.56 (m, 2H), 2.20 (m, 2H), 2.18 (m, 2H), 1.82-1.50 (m, 2H), 1.79 (t, 3H, J = 7.2 Hz), 1.68 (d, 3H, J = 7.5 Hz), -0.13 (brs, 1H), -2.07 (brs, 1H); Anal. Calcd for $C_{41}H_{42}N_4O_5$: C, 73.40; H, 6.32; N, 8.35. Found: C, 73.56; H, 6.51; N, 8.53%.