Synthesis of Chlorins Linked with Pyrazoline
by 1,3-Dipolar Diazomethane Cycloaddition Reaction

Jin-Jun Wang,[†] Jia-Zhu Li,[†] Daniel Gryko,[‡] and Young Key Shim[*]

[†]*Department of Applied Chemistry, Yantai University, Yantai 264005, China*
[‡]*Institute of Organic Chemistry, Polish Academy of Science, Warsaw 01-224, Poland*
[*]*PDT Research Institute, School of Nano Engineering, Inje University, Gimhae 621-749, Korea. [*]E-mail: ykshim@inje.ac.kr*
*Received May 12, 2006*

Chlorins and bacteriochlorins, occurred in natural systems as a class of tetrapyrrolic macrocycles, have gained much interests in recent years because of their unique optical and photochemical properties. They can be utilized as the second generation photosensitizers for photodynamic therapy (PDT) of cancer[1] and as models for photosynthetic reaction centers.[2] Among the chlorophyll derivatives, pheophorbide, pyropheophorbide, purpurin and *N*-alkylimide of purpurin-18 have been reported as potent photosensitizers with significant anticancer activity.[3-5] Their derivatives were usually prepared by chemical modification of peripheral functional groups, particularly the vinyl group at 3-position.[6] Our interest in the synthesis of chlorins as potential photosensitizers for PDT led us to explore new strategies for vinyl functionalization of pheophorbide-a by 1,3-dipolar

cycloaddition methodology with diazomethane.

1,3-Dipolar [3+2] cycloaddition is an effective method for the synthesis of five-membered heterocycles. It is well known that diazomethane is a reactive reagent for 1,3-dipolar cycloaddition with olefin to furnish pyrazoline derivatives, which serve as useful building blocks in the synthesis of various compounds through chemical transformation and/or ring cleavage. In the present work, the novel pyrazolinyl chlorins, promising versatile starting materials for further application by chemical transformation of pyrazoline ring for constructing new structure of chlorin, were synthesized by 1,3-dipolar cycloaddition reaction with chlorin and diazomethane. It was found that the vinyl group of pheophorbide-a **1**, extracted from the alga *Spirulina maxima*,[7] reacts slowly over a period of 3 days with ethereal
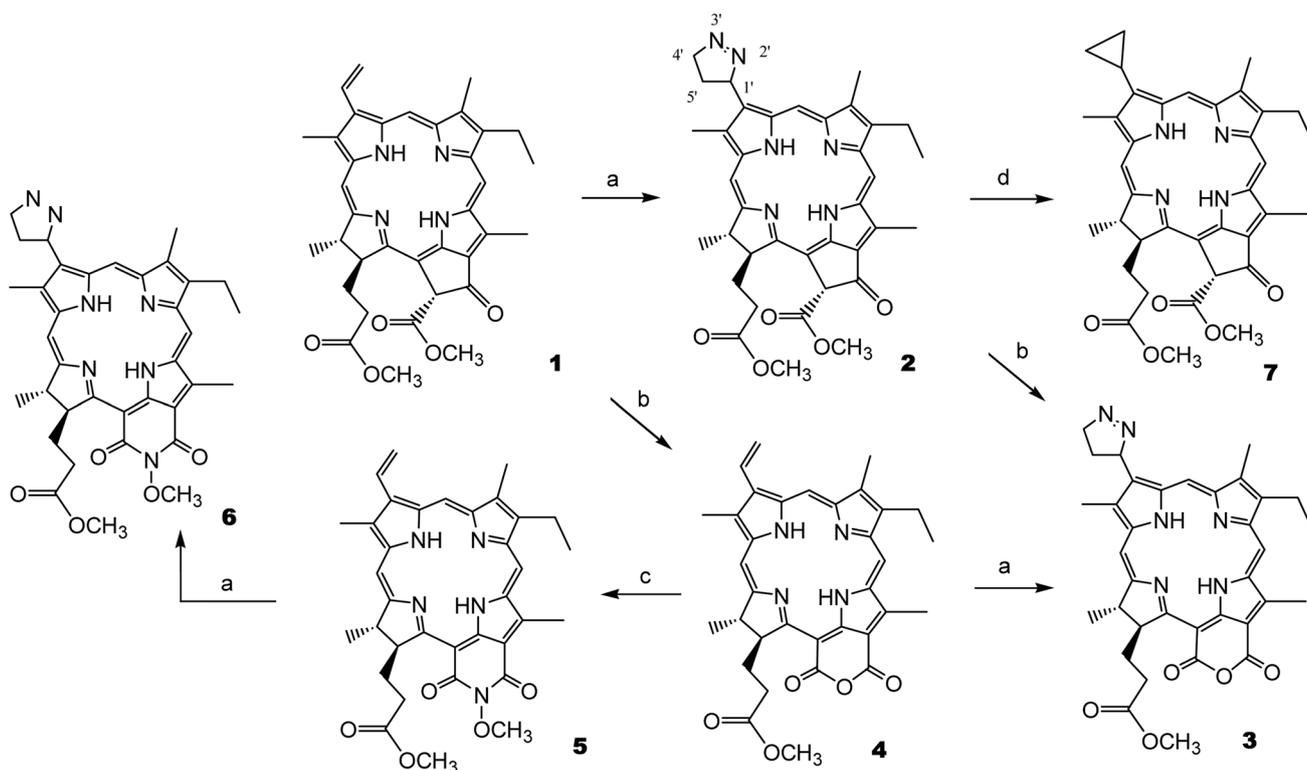
Scheme 1. a) CH_2N_2/CH_2Cl_2 (**2**: 65%; **3**: 58%; **6**: 54%); b) $O_2/KOH/CH_3CH_2CH_2OH/Py/CH_2N_2$ (**3**: 9%; **4**: 68%); c) $NH_2OH \cdot HCl/Py$ (**5**: 90%); d) *o*-DCB/180 °C (**7**: 80%).
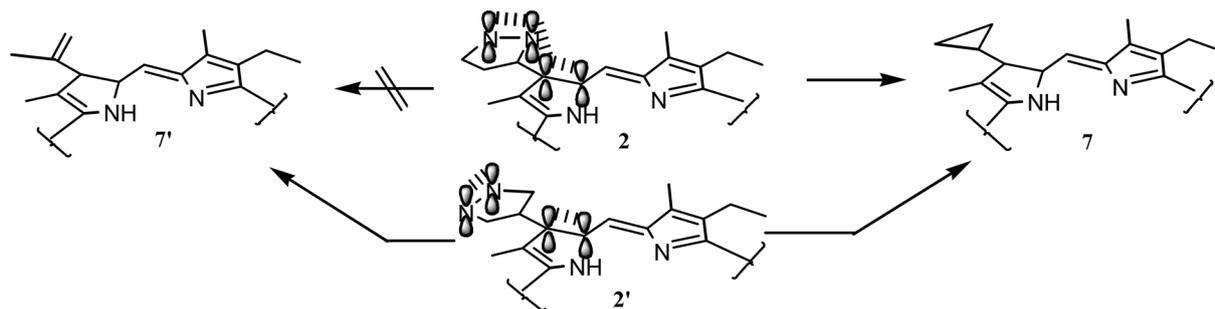
Figure 1. The difference between chlorin **2** and **2'** in homoconjugation effect with parent ring and their ring cleavage.

diazomethane in dichlormethane to produce a single pyrazolinyl-substituted chlorin **2** as a 1 : 1 3-epimeric mixture in 65%, which was easily isolated via chromatography on silica. Molecular structure of this pyrazolyl chlorin was determined by its 1D ^{1}H NMR and 2D/ROESY ^{1}H NMR to present two (R,S)-epimers at the 3^1-chiral center. The cyclic product **2** was transformed into purpurin-18 derivative **3** by aeration in propanol with KOH in the presence of pyridine in low yield (9%). The purpurin-18 methyl ester **4**, obtained from starting chlorin **1** by the same air-oxidation, was converted into the chlorin **3** by 1,3-dipolar cycloaddition reaction with diazomethane in 58% yield. The reaction of purpurin-18 methyl ester **4** with excess hydroxyl-amine in pyridine at room temperature afforded purpurin-18 *N*-hydroxyimide **5** (90%). Using a similar methodology, treatment of chlorin **5** with diazomethane gave 3-pyrazo-linyl-substituted purpurinimide **6** in 54% yield.

To test the chemical reactivity of the pyrazolinyl group, 3-devinyl-3-[3'(R,S)-(1'-pyrazolinyl) pheophorbide **2** refluxed in *o*-dichlorobenzene smoothly changed to the cyclopropyl analog **7** as an only product with an excellent yield (90%). This result further indicated that the 1,3-dipolar cyclo-addition of vinyl chlorin gave an anti-Markovnikov type product **2** without any opposite regioselective product **2'**. (Figure 1) This Markovnikov type compound will give two ring-cleaved products (**7** and **7'**) similar to the regioselective 1,3-dipolar cycloaddition of styrene with diazomethane.[8]

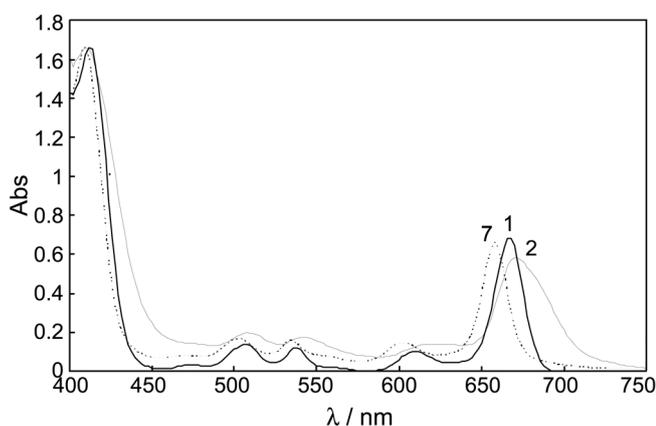Further evidence for regioselectivity came from the

absorption spectra, where the peak at 670 nm of chlorin **2** was slightly red-shifted compare to the corresponding peak (666 nm) of the starting material **1**. The longest absorption band of chlorophyll derivatives, having no conjugated structure of the parent ring at 3-position, generally appeared below 660 nm such as the chlorin **7** which has Qy absorption at 658 nm.[9] The Qy-peak of chlorin **2**, showed at 670 nm, is ascribed to the homoconjugation effect between N=N double bond in the pyrazoline ring at 3-position with chlorin chromophore, whereas this relationship was not monitored for the isomer **2'**. The similar result was also discovered for purpurin imide derivatives **4** (698 nm)/**3** (708 nm) and **5** (708 nm)/**6** (720 nm).

In conclusion, the synthetic methodology utilizing 1,3-cycloaddition of vinyl-substituted chlorins possessing chloro-phyll-*a* skeleton with diazomethane lays the groundwork for important peripheral functionalization of tetrapyrrolic ring systems. Such reactions constructed conveniently five-membered heterocycles and three-membered cycloalkyl moieties on the macrocycles, and these modifications for parent ring of chlorophyll-*a* derivatives may be valuable in the generation of novel photosensitisers for PDT.

Experimental Section

General. The reactions were monitored by TLC using silica gel plates and the products were purified by flash column chromatography on silica gel (Merck; 230-400 mesh). The ^{1}H NMR spectra were recorded on a Varian-400 MHz spectrometer. Chemical shifts are given as δ values using tetramethylsilane as the internal standard and *J* values in Hz. Elemental analysis were performed on a Perkin-Elmer 240-C microanalyzer. All chemical reagents were commercially available and purified with standard methods before use. Methyl pyropheophorbide-*a* **1** was prepared according to Smith's method[7]. Purpurin-18 methyl ester **4** and **5** were obtained according the reference 9.

3-Devinyl-3-[3'(R,S)-(1'-pyrazolinyl0)]pheophorbide-*a* methyl ester 2. The chlorin **1** (100 mg, 0.165 mmol) was dissolved in dichloromethane (60 mL) and an ethereal solution of diazomethane (40 mL), prepared from diazold (1.8 g), was added to the reaction mixture. The resulting solution was sealed with a rubber septum and kept at room temperature for 72 h. The solvent was degassed by bubbling

Figure 2. UV-vis spectra of **1**, **2** and **7** in CHCl₃.

with nitrogen and the resulting solution was evaporated in vacuum. The residue was purified by column chromatography on silica gel with hexane-ethyl acetate (4 : 1) to give **2** (70 mg, 65%) as a green solid; m.p. 221-223 °C; UV-vis (CHCl$_3$) λ_{max}: 411 (9.86 × 10^4), 508 (1.28 × 10^4), 541 (1.09 × 10^4), 618 (8.88 × 10^3), 670 (3.65 × 10^4) nm; ^{1}H NMR (400 MHz, CDCl$_3$, δ): −1.71 (br s, 1H, NH), 0.40 (br s, 1H, NH), 1.68 (t, J = 7.5 Hz, 8$_b$-CH$_3$), 1.82 (d, 3H, J = 7.3 Hz, 18-CH$_3$), 2.10-2.18 (m, 1H, 4'$_b$-H), 2.22-2.39, 2.47-2.69 (each m, 2H, 17$_a$+17$_b$-H), 2.74-2.83 (m, 1H, 4'$_a$-H), 3.16 (s, 3H, 7-CH$_3$), 3.27 (s, 3H, 2-CH$_3$), 3.58 (s, 3H, 17-OCH$_3$), 3.66 (q, J = 7.5 Hz, 8$_a$-CH$_3$), 3.68 (s, 3H, 12-CH$_3$), 3.89 (s, 3H, 13^2-OCH$_3$), 4.20-4.27 (m, 1H, 17-H), 4.43-4.53 (m, 1H, 18-H), 4.78 (dtd, 1H, J = 16.0, 6.5, 2.4 Hz, 5'$_b$-H), 5.50 (ddt, 1H, J = 16.0, 5.16, 2.4 Hz, 5'$_a$-H), 6.27 (s, 1H, 13^2-H), 6.67 (tt, J = 12.5, 2.8 Hz, 3'-H), 8.61 (s, 1H, 20-H), 8.98, 8.97 (two s, each 0.5H, 5-H), 9.52 (s, 1H, 10-H). Anal. Calcd for C$_{37}$H$_{40}$N$_6$O$_5$: C, 68.50, H, 6.21, N 12.95; found C, 68.62, H 6.43, N, 12.74.

3-Devinyl-3-[3'(R,S)-(1'-pyrazolinyl)]-*N*-methoxyl pur-purin-18 imide methyl ester (6). This chlorin was synthesized in 54% yield from **5** by the similar method for **2**. m.p. 231-233 °C; UV-vis (CHCl$_3$) λ_{max} (ε): 366 (6.58 × 10^4), 418 (1.10 × 10^5), 508 (8.78 × 10^3), 560 (2.30 × 10^4), 720 (3.95 × 10^4) nm; ^{1}H NMR (400 MHz, CDCl$_3$, δ): −0.07 (br s, 1H, NH), −0.01 (br s, 1H, NH), 1.58 (t, J = 7.5 Hz, 8$_b$-CH$_3$), 1.76 (d, 3H, J = 7.4 Hz, 18-CH$_3$), 1.98-2.18 (m, 1H, 4'$_b$-H), 2.20-2.15, 2.37-2.57 (each m, 2H, 17$_a$+17$_b$-H), 2.72-2.88 (m, 1H, 4'$_a$-H), 3.01 (s, 3H, 7-CH$_3$), 3.21 (s, 3H, 2-CH$_3$), 3.47 (q, J = 7.5 Hz, 8$_a$-CH$_3$), 3.60 (each s, 3H, 17-OCH$_3$), 3.67 (s, 3H, 12-CH$_3$), 4.39 (each s, 3H, N-OCH$_3$), 4.35-4.45 (m, 1H, 18-H), 4.76 (td, 1H, J = 18.0, 8.9 Hz, 5'$_b$-H), 5.30 (dd, 1H, J = 8.3, 16.8 Hz, 17-H), 5.45 (dd, 1H, J = 18.0, 9.8, Hz, 5'$_a$-H), 6.55 (dd, J = 12.5, 7.8, Hz, 3'-H), 8.58 (s, 1H, 20-H), 8.93, 8.90 (two s, each 0.5H, 5-H), 9.36 (s, 1H, 10-H). Anal. Calcd for C$_{36}$H$_{39}$N$_7$O$_5$: C, 66.55, H, 6.05, N 15.09; found C, 66.32, H 6.23, N, 15.24.

3-Devinyl-3-cyclopropylpheophorbide-*a* methyl ester 7. The chlorin **2** (100 mg, 0.154 mmol) was dissolved in *o*-dichlorobenzen (40 mL) and refluxed for 20 min. The reaction solution was diluted with hexane (150 mL), filtered and purified by column chromatography on silica gel with hexane-ethyl acetate (3 : 1) to give **7** (77 mg, 80%) as a green solid; m.p. 197-199 °C; UV-vis (CHCl$_3$) λ_{max} (ε): 410 (1.04 × 10^5), 471 (5.18 × 10^3), 504 (1.14 × 10^4), 534 (1.04 × 10^4), 603 (9.43 × 10^3), 658 (4.69 × 10^4) nm; ^{1}H NMR (400 MHz, CDCl$_3$, δ): −1.61 (br s, 1H, NH), 0.82 (br s, 1H, NH), 1.28-1.36 (m, 2-H, 3$_b^2$-H+ 3$_b^3$-H), 1.57-1.68 (m, 2-H, 3$_a^2$-H+3$_a^3$-H), 1.70 (t, J = 7.6 Hz, 8$_b$-CH$_3$), 1.79 (d, 3H, J = 7.3 Hz, 18-CH$_3$), 2.45-2.68, 2.18-2.38 (each m, 3H, 17$_a$+17$_b$-H) 2.74-2.84 (m, 2H, 3$_1$-H+17$_a$-H), 3.24 (s, 3H, 7-CH$_3$), 3.58 (s, 3H, 17-OCH$_3$), 3.67 (s, 3H, 12-CH$_3$), 3.68 (q, J = 7.5 Hz, 8$_a$-CH$_3$), 3.88 (s, 3H, 13^2-OCH$_3$), 4.16-4.22 (m, 1H, 17-H), 4.38-4.47 (m, 1H, 18-H), 6.24 (s, 1H, 13^2-H), 8.45 (s, 1H, 20-H), 9.47 (s, 1H, 5-H), 9.56 (s, 1H, 10-H);. Anal. Calcd for C$_{37}$H$_{40}$N$_4$O$_5$: C, 71.59, H, 6.50, N 9.03; found C, 71.36, H 6.73, N, 9.24.

References

1. (a) Nyman, E. S.; Hynninen, P. H. *J. Photochem. Photobiol. B* **2004**, *73*, 1-28. (b) Bourre, L.; Simonneaux, G.; Farand, Y.; Thibautt, S.; Lajat, Y.; Patrice, T. *J. Photochem. Photobiol. B* **2003**, *69*, 179-192.
2. (a) Ohkubo, K.; Kotani, H.; Shao, J. G.; Ou, Z. P.; Kadissh, K. M.; Li, G. L.; Pandey, R. K.; Fujitsuka, M.; Ito, O.; Imahori, H.; Fukuzumi, S. *Angew. Chem., Int. Ed.* **2004**, *43*, 853-853. (b) Vlassiouk, I.; Smirov, S.; Kutzki, O.; Wedel, M.; Montforts, F. P. *J. Phys. Chem. A* **2002**, *106*, 10991-10998.
3. Pandey, R. K.; Jagerocic, N.; Ryan, J. M.; Dougherty, T. J.; Smith, K. M. *Tetrahedron* **1996**, *52*, 5349- 5362.
4. Kozyrev, A. N.; Zheng, G.; Zhu, C.; Doughrty, T. J.; Smith, K. M.; Pandey, R. K. *Tetrahrdon Lett.* **1996**, *37*, 6431-6434.
5. Garbo, G. *J. Photochem. Photobiol. B: Biology* **1996**, *34*, 109-116.
6. (a) Kunieda, M.; Mizoguchi, T.; Tamiaki, H. *Tetrahedron* **2004**, *60*, 11349-11357. (b) Tamiaki, H.; Kouraba, M. *Tetrahedron* **1997**, *53*, 10677-10688. (c) Lee, J.-C.; Kim, T.-Y.; Kang, S. H.; Shim, Y. K. *Bull. Korean Chem. Soc.* **2001**, *22*, 257-258.
7. Smith, K. M.; Goff, D. A.; Simposon, D. G. *J. Am. Chem. Soc.* **1985**, *107*, 4946-4954.
8. Husigen, R. In *1,3-Dipolar Cycloaddition Chemistry*; Padwa, A., Ed.; Wiley-Interscience: New York, 1984.
9. Mironov, A. F.; Lebedeva, V. S. *Tetrahedron Lett.* **1998**, *39*, 905-908.