Model Studies in Photosynthesis (I). Synthesis and Characterization of Some Novel Pyropheophorbide Derivatives

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2′-(9″-Anthracenecarbonyloxy)ethyl pyropheophorbide *a*, methyl 3a-(9′-anthracenecarbonyloxy)pyropheophorbide *a*, 2′-(4″-phenylbenzoyloxy)ethyl pyropheophorbide *a*, and methyl 3a-(4′-phenylbenzoyloxy)pyropheophorbide *a* were prepared from chlorophyll *a* and *b*. Nuclear magnetic resonance study showed that the sandwich conformation is more favorable in 2′-(9″-anthracenecarbonyloxy)ethyl pyropheophorbide *a* than it is in other compounds.

Introduction

Photosynthesis[1] is the process by which chlorophyll-containing plants convert solar energy into photochemical energy. Three main groups of photosynthetic pigments are known: chlorophylls, carotenoids, and phycobilins. The function of these pigments is to provide the plants with an efficient system of absorbing light throughout the visible spectrum.

The existence of photosynthetic units allows a more efficient use of the absorbed energy. This involves excitation energy migration through a maze of several hundred Chl *a* molecules, until the energy finally reaches the reaction center where it is converted into chemical energy. A special dimer of Chl *a*-H_2O and a molecule of pheophytin a were suggested to be in the reaction centers of photosystem I.[2]

The process of excitation energy migration through the same kind of molecules involves homogeneous energy transfers. In addition the energy absorbed by pigments other than Chl *a* in any photosystem is also transferred to Chl *a*. This kind of excitation energy transfer between different kinds of pigments is called heterogeneous energy transfer. Evidence for the existence of heterogeneous energy transfer comes from fluorescence measurements. The excitation spectrum of Chl *a* fluorescence should theoretically follow the absorption spectrum of Chl *a* if there is no heterogeneous transfer; however, it remains high even in the regions of low Chl *a* absorption. This is particularly striking in the blue-green and red algae, where the action spectrum of Chl *a* fluorescence is very high in the regions of phycocyanin and phycoerythrin absorption.

Considerable overlap of the absorption band of the acceptor molecule, in this case Chl *a*, and the fluorescence band of the donor molecules (Chl *b* or phycoerythrin and phycocyanin), the appropriate orientation and the close distance of these pigments are the important conditions for the high efficiency of energy transfer.

To study geometric requirements in energy transfers between chlorophyll and other chromophores, we prepared pyropheophorbide a derivatives with covalently-linked anthracenecarboxylate and 4-phenylbenzoate functionalities and their solution conformations were studied with the aid of nmr spectroscopic methods. The results of the spectrofluorometric studies will be reported spearately.[3] Geometric requirements for the mimicry of antenna and photoprotective carotenoid functions by synthetic carotenoporphyrins were recently discussed by other workers.[4~6]

Results and Discussion

Synthesis. Demetallation of chlorophylls was carried out by passing dry hydrogen chloride gas through the crude chlorophyll extract in hexane-ether solution (1:1, v/v) and and quenching excess hydrogen chloride by addition of solid potassium monohydrogen phosphate yielding mixture of pheophytins. In the absence of ether, phytyl ester bond was also attacked, and free pheophorbides separated from the solution.

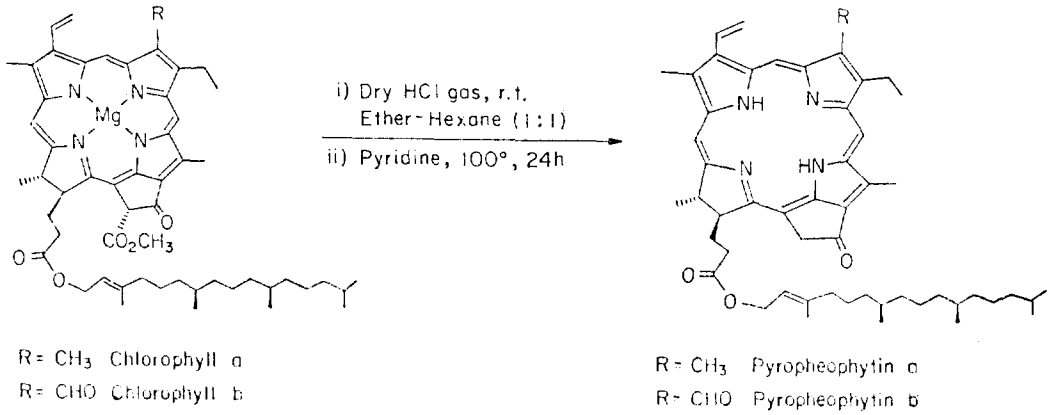
Pheophytins were separated by column chromatography to yield pheophytins *a* and *b*. Pyropheophytin *a*(**1**) was prepared from pheophytin a in hot pyridine and methyl pyropheophorbide *a*(**2**) was obtained by subsequent methanolysis (Schemes 1 and 2).

Pyropheophytin *a*(**1**) was also converted to 2-hydroxyethyl pyropheophorbide *a*(**3**) in ethylene glycol-sulfuric acid and 2′-(9″-anthracenecarbonyloxy)ethyl pyropheophorbide *a*(**4**) was obtained by esterification of **3** with 9-anthracenecarbonyl chloride and 4-(dimethylamino)pyridine in pyridine (Scheme 3).

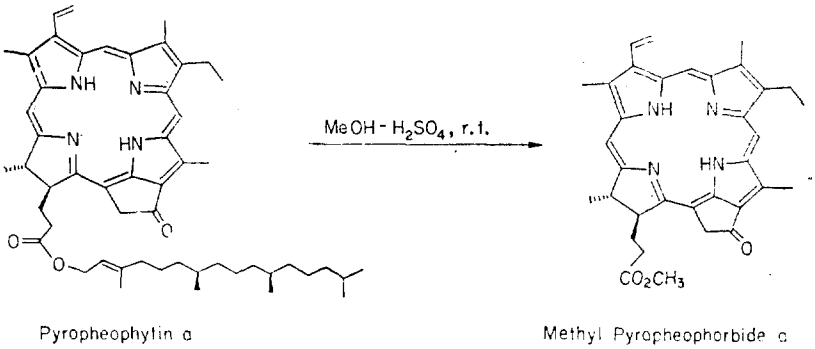
Coupling between free pyropheophorbide a and anthracenemethanol was unsuccessful using dicyclohexylcarbodiimide, 2-chloro-1-methylpyridinium iodide in methylene chloride, or 1,1′-carbonyldiimidazole in THF.

Pyropheophytin *b*(**5**) was prepared from pheophytin *b* in the same way and converted to methyl pyropheophorbide *b*(**6**). The formyl group of **6** was selectively reduced with sodium cyanoborohydride and 18-crown-6 in ether-absolute ethanol to yield methyl 3a-hydroxypyropheophorbide *a*(**7**) in good yields. Esterification of **7** with 9-anthracenecarbonyl chloride and 4-(dimethylamino)pyridine in pyridine produced methyl 3a-(9′-anthracenecarbonyloxy) pyropheophorbide *a*(**8**) as expected (Scheme 4).

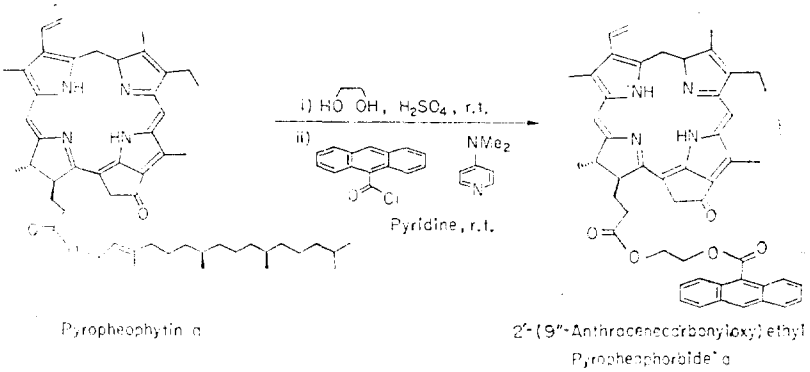
Use of 4-phenylbenzoyl chloride in place of 9-anth-



Scheme 1.



Scheme 2.

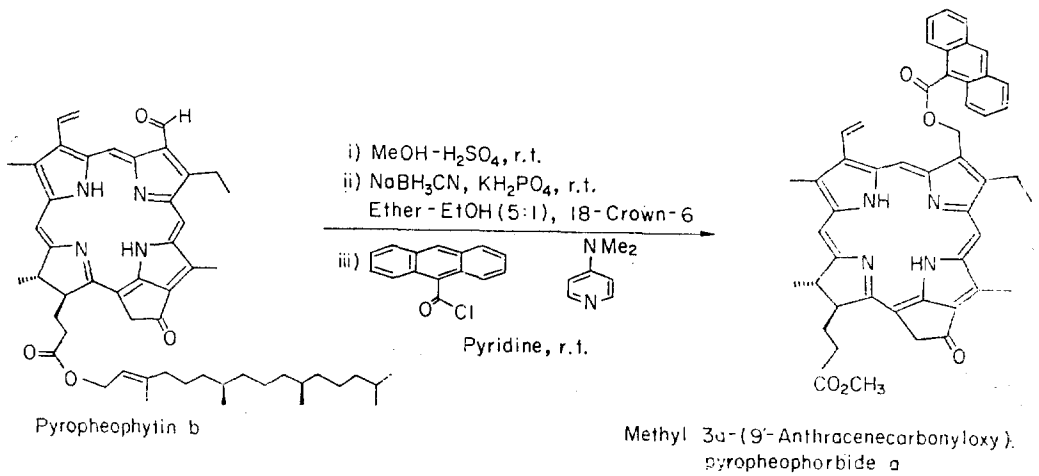


Scheme 3.

racenecarbonyl chloride in each synthetic scheme enabled the synthesis of 2'-(4''-phenylbenzoyloxy)ethyl pyropheophorbide *a* (**9**) and methyl 3a-(4'-phenylbenzoyloxy)pyropheophorbide *a* (**10**).

Nuclear Maqnetic Resonance Study. The nmr spectra(in $CDCl_3$) of methyl pyropheophorbide *a*(**2**), 2'-(9''-anthracenecarbonyloxy) ethyl pyropheophorbide *a*(**4**), methyl 3a-(9'-anthracenecarbonyloxy)pyropheophorbide *a*(**8**), 2'-(4''-phenylbenzoyloxy)ethyl pyropheophorbide *a*(**9**), and methyl 3a-(4'-phenylbenzoyloxy)-pyropheophorbide *a*(**10**) are presented in Figures 1,2,3,4, and 5. The chemical shift data are summarized in Table 1 according to the standard numbering system. (Figure 6) The data for methyl 9-anthracenecarboxylate (**11**) and methyl 4-phenylbenzoate (**12**) are also listed for comparison.

Comparison of pyropheophorbide proton chemical shifts of **4** with those of **2** indicates that the region between α-H and β-H (ring B region) is noticeably deshielded and the



Scheme 4.

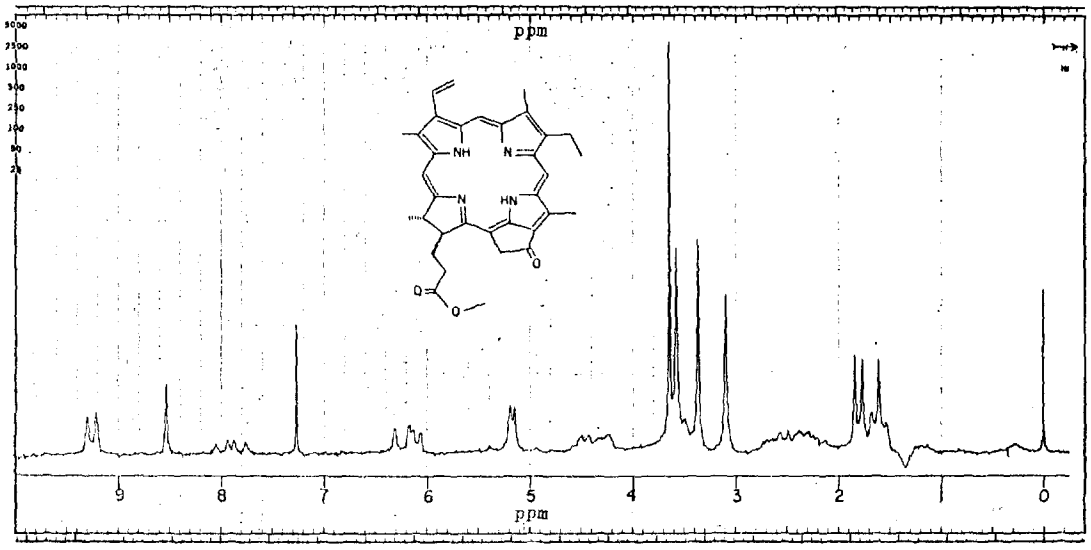


Figure 1

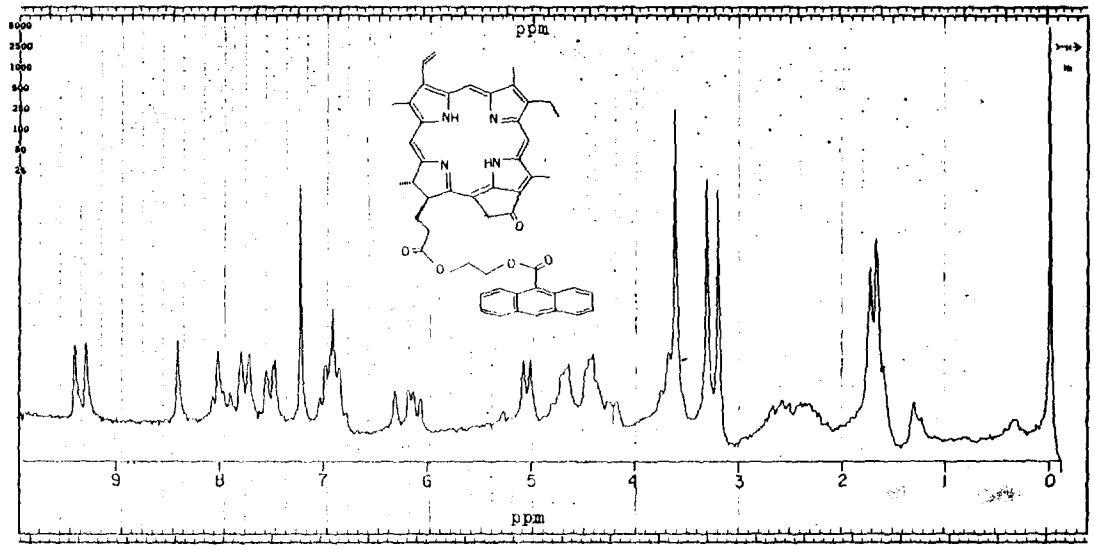


Figure 2

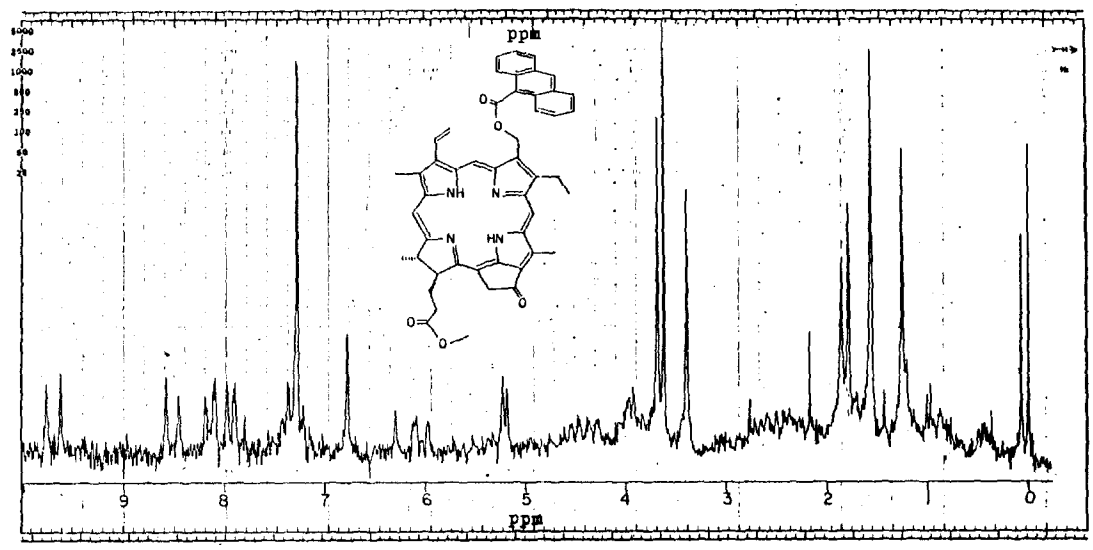


Figure 3

region between 1a-H and 10-H (ring D region) is shielded. On the other hand, much less magnetic anisotropic effect is noticed when the chemical shift values for pyropheophorbide protons of **9** are compared with those of **2** (Table 2). It can thus be inferried that the sandwich conformation of **4** in which the anthracene ring sits on top of ring D

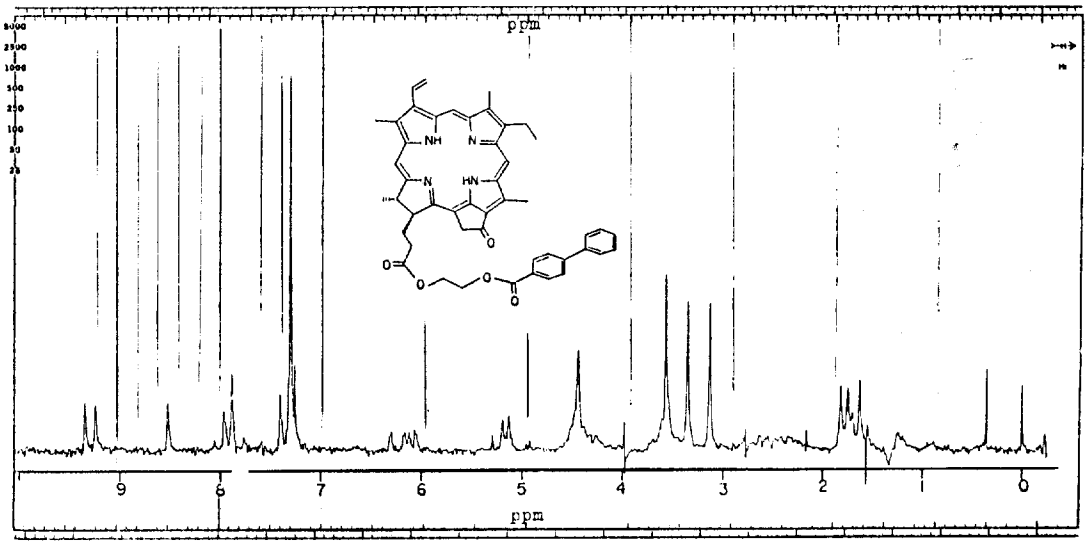


Figure 4

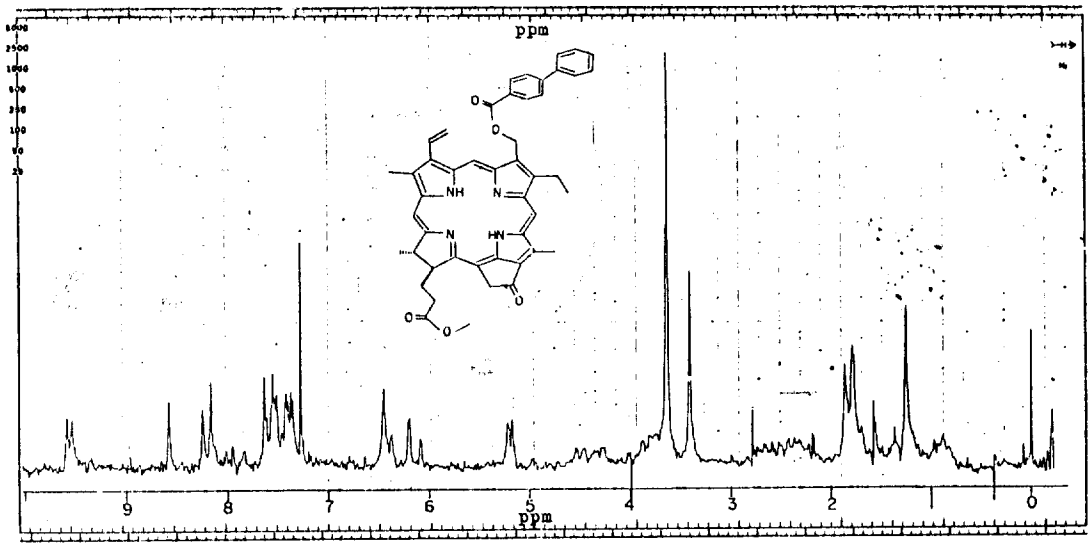


Figure 5

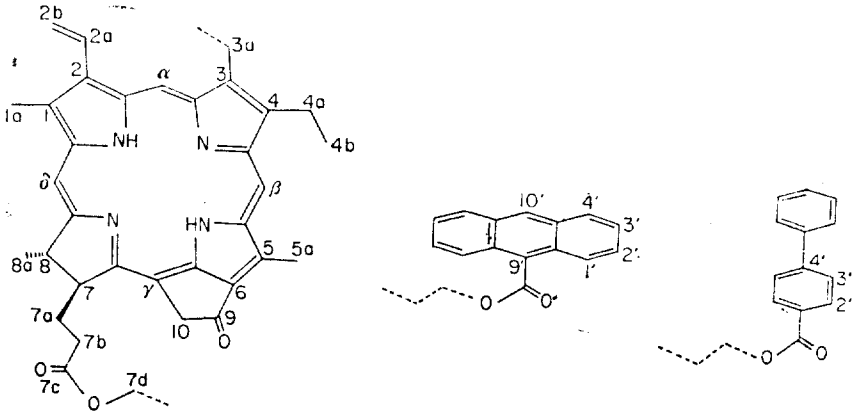


Figure 6

contributes to some degree under the nmr measurement conditions, whereas the same type of conformation is less favorable for **9**. Apparently, the anthracenecarboxylate chromophore interacts more favorabtly with the pyropheophorbide ring system than the 4-phenylbenzoate chromophore does under the same circumstances.

The same conclusion can be reached when one examines the proton chemical shift values of anthracenecarboxylate and 4-phenylbenzoate in **4** and **9**. The anthracene ring protons of **4** are strongly shielded compared to those of **11**. The shielding effect on the protons of the biphenyl system in **9** compared to the protons of **12** appears to be much smaller (Table 1).

Next, nmr data of **8** and **10** are examined. Protons in the regions between α-H and β-H (ring B region) are strongly deshielded in both **8** and **10** compared with those of **2**, and it can be concluded that the same type of conformation is predominant for **8** and **10**, in which the pyropheophorbide chromophore lies in the deshielding region of anthracenecarboxylate or 4-phenylbenzoate moiety (Table 3). The only discrepancy with 2-H is probably due to the different shape of each ring system.

Conclusion. From the close examination of nmr data, it is concluded that relative geometry of the two different chromophores in **8** and **10** is quite different from those in **4** and **9**. The contribution of the sandwich conformation

TABLE 1: Proton NMR Chemical Shifts for Pyropheophorbide Derivatives (δ)

Proton	(2)	(4)	(8)	(9)	(10)
1a	3.36	3.32	3.40	3.36	3.40
2a	7.89	7.97			
2b(*E*)	6.11	6.15	6.02	6.11	6.13
2b(*Z*)	6.21	6.24	6.19	6.20	6.27
α	9.21	9.34	9.57	9.20	9.48
3a	3.09	3.21	6.76	3.14	6.44
4b	1.60	1.68	1.79	1.64	1.78
β	9.29	9.45	9.71	9.31	9.63
5a	3.57	3.63	3.69	3.57	3.64
10	5.16	5.06	5.19	5.16	5.18
7d	3.64		3.62		3.62
8a	1.80	1.71	1.83	1.79	1.83
δ	8.52	8.45	8.54	8.49	8.54
	(11)	(4)	(8)		
1′,4′	8.07	7.79	8.11		
		7.55	7.91		
2′,3′	7.55	6.95	7.28		
10′	8.53	8.06	8.42		
	(12)			(9)	(10)
2″	8.14			7.91	8.17
3″	7.68			7.36	7.57
Ph–	≈7.5			7.30	7.3≈7.6

TABLE 2: Difference in Proton NMR Chemical Shifts (δ)

Proton	(2)–(4)	(2)–(9)
1a	–0.04	0
2b(*E*)	–0.04	0
2b(*Z*)	+0.03	–0.01
α	+0.13	–0.01
3a	+0.12	+0.05
4b	+0.08	+0.04
β	+0.16	+0.05
5a	+0.06	0
10	–0.10	0
8a	–0.09	–0.01
δ	–0.07	–0.03

TABLE 3: Difference in Proton NMR Chemical Shifts (δ)

Proton	(2)–(8)	(2)–(10)
1a	+0.04	+0.04
2b(*E*)	–0.09	+0.02
2b(*Z*)	–0.02	+0.06
α	+0.36	+0.27
4b	+0.19	+0.18
β	+0.42	+0.34
5a	+0.12	+0.07
10	+0.03	–0.02
7d	–0.02	–0.02
8a	+0.03	+0.03
δ	+0.02	+0.02

is greater in **4** than in **9**. When a chromophore is attached *via* 3a-carbonyloxy bridge, pyropheophorbide ring lies in the deshielding region of the chromophore, as in **8** and **10**.

It will be interesting to see how these geometric factors affect the efficiency in the excitation energy transfer. The results of spectrofluorometric analyses will be reported in due courese.

Experimental

Crude reaction mixtures were always checked with the thin layer chromatography. TLC plates were made by dipping microscopic slides in the chloroform–methanol (2:1) slurry of silica gel (Merck Art. 7731 Kieselgel type G60) or cellulose (Merck Art. 2330 Cellulose Microcrystalline Avicel) and drying in the air.

Nuclear magnetic resonance spectra were determined by using a Varian XL-100 FT system with tetramethylsilane as an internal standard in deuterochloroform.

Preparation of Chlorophylls. The crude chlorophyll extract was prepared from spinach leaves by the method of Strain and Svec.[7]

Preparation of Pheophytins. The same volume of ether was added to the crude chlorophyll extract in hexane. Dry hydrogen chloride gas was passed briefly through the solution until the color changed from green to brown. Solid K_2HPO_4 was added to the crude pheophytin solution to quench the excess hydrogen chloride gas.

Separation of Pheophytins. The crude pheophytin solution was filtered and concentrated. The concentrated solution was separated on a silica gel column. (Merck Art. 7734 Kieselgel G 60, 2% acetone in carbon tetrachloride or 10% acetone–hexane)

TLC: cellulose, petroleum ether–acetone 6:1 pheophytin *a*, R_f 0.92; pheophytin *b*, R_f 0.68

Pyropheophytin a(**1**). Pheophytin a (1.83 g) was dissolved in 300 m*l* of pyridine. The resulting mixture was heated at 100° under nitrogen atmosphere. After 24 hours, the crude product was poured into 600 m*l* of ether. The ether solution was washed several times with 2 N HCl solution, once with saturated sodium bicarbonate solution and once with saturated sodium chloride solution. The organic layer was separated and dried over anhydrous sodium sulfate. After solvent removal, 1.61 g of pyropheophytin *a* was obtained. Yield 95 %. TLC: silica gel, petroleum ether–acetone 4:1, R_f 0.61.

Methyl Pyropheophorbide a (**2**). Pyropheophytin *a* (220 mg) was dissolved in methanol (50 m*l*), and 2 m*l* of concentrated sulfuric acid was added slowly. The mixture was stirred under nitrogen atmosphere at room temperature for 24 hours. The solution was diluted with ether, washed three times with water and twice with saturated sodium chloride solution. The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated to yield 92 mg of the product. Yield 70 %. The product was crystallized in benzene–petroleum ether. TLC: cellulose, petroleum ether–acetone 6:1, R_f 0.73.

2-Hydroxyethyl Pyropheophorbide a(**3**). Pyropheophytin a (500 mg) was dissolved in excess ethylene glycol containing catalytic amount of concentrated sufuric acid. The mixture was stirred for a day under nitrogen atmosphere at room temperature. The crude product was worked up in a standard way. After the solvent removal, 200 mg of the crude product

was obtained. Yield 62 %. The crude product was crystallized in benzene-petroleum ether. TLC: cellulose, petroleum ether-acetone 5:1, R_f 0.45.

2'-(9''-Anthracenecarbonyloxy)ethyl Pyropheophorbide a(**4**). To a solution of **3** (100 mg) in pyridine (20ml), excess 9-anthracenecarbonyl chloride and catalytic amount of 4- (dimethylamino)pyridine were added. The mixture was stirred under nitrogen atmosphere at room temperature. After two days, the crude product was poured into 150 m*l* of ether and the mixture was washed with 2 *N* HCl three times and once with water. Some N,N-dimethylaminopropylamine was added into the resulting solution in order to remove unreacted anthracene derivatives. After 30 minutes, the solution was washed with 2 *N* HCl for several times, three times with saturated sodium bicarbonate solution, and dried over anhydrous sodium sulfate. The filtrate was concentrated and the product was purified on a silica gel column to yield 42 mg of the solid. Yield 30 %. It was crystallized in methylene chloride-petroleum ether. TLC: cellulose, petroleum ether-acetone 6:1, R_f 0.55.

Pyropheophytin b(**5**). Pyropheophytin b was prepared from pheophytin b using the same procedure as with pyropheophytin a. Yield 84 %. TLC: silica gel, petroleum ether-acetone 4:1, R_f 0.54.

Methyl Pyropheophorbide b(**6**). Methyl pryopheophorbide *b* was prepared by the same procedure used for the preparation of methyl pyropheophorbide *a*. Yield 76 %. The product was crystallized in benzene-petroleum ether. TLC: cellulose, petroleum ether-acetone 6:1, R_f 0.60.

Methyl 3a-Hydroxypyropheophorbide a(**7**). The solution of **6** (120 mg) in ether-absolute ethanol (5:1) was treated with excess solid KH_2PO_4, excess sodium cyanoborohydride, and catalytic amount of dicyclohexyl-18-crown-6. The suspension was stirred under nitrogen atmosphere at room temperature for two days. The reaction mixture was washed three times with water and twice with saturated sodium chloride solution. The organic layer was dried over anhydrous sodium sulfate, filtered, and evaporated. Purification on a silica gel column produced 80 mg of the solid. Yield 66 %. The product was crystallized in methylene chloride-petroleum ether. TLC: cellulose, petroleum ether-acetone, 5:1, R_f 0.45.

Methyl 3a-(9'-Anthracenecarbonyloxy)pyropheophorbide a(**8**). It was prepared from **7** under the same conditions as in the synthesis of **4**. The product was crystallized in methylene chloride-petroleum ether. TLC: cellulose, petroleum ether-acetone 6:1, R_f 0.55.

2'-(4''-Phenylbenzoyloxy)ethyl Pyropheophorbide a(**9**) & *Methyl 3a-(4'-phenylbenzoyloxy)pyropheophorbide a*(**10**). Esterification of **3** and **7** with 4-phenylbenzoyl chloride using the procedure adopted in the synthesis of **4** produced **9** and **10** in comparable yields.

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