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Chlorophylls. VIII. Synthesis of a New Chlorophyll Derivative and Anthraquinone-Based Dyad and Triad Molecules for Executing Photoinduced Electron Transfer

Andrei Y. Tauber,[a] Juho Helaja,[a,b] Ilkka Kilpeläinen[b] and Paavo H. Hynninen[a,*]

[a]Department of Chemistry, P. O. Box 55, University of Helsinki, FIN-00014 Helsinki, Finland and [b]Institute of Biotechnology, P.O. Box 45, University of Helsinki, FIN-00014 Helsinki, Finland

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A new chlorophyll derivative and anthraquinone based dyad and triad model compounds have been synthesized. Trifluoroacetic anhydride is shown to be an efficient reagent for ether bond formation between 3^1-hydroxyphytochlorin and 2-(hydroxymethyl)anthraquinone. The application of Mukaiyama's reagent for the final esterification allowed us to create quite complex molecules under very mild conditions. All diastereomers of the new dyads and triads were successfully separated using HPLC techniques.

A basic challenge of artificial photosynthesis studies is to design and prepare synthetic systems that mimic natural photosynthesis. Since the X-ray diffraction data of the photosynthetic reaction centre (RC) complexes from *Rhodopseudomonas viridis*[1–3] and *Rhodobacter spheroides*[4,5] were reported, a great number of model systems have been created and studied regarding the modelling of the primary steps of electron transfer in the RC.[6,7] However, in spite of the great number of models, only a few pheophorbide–quinone dyads based on natural chlorin chromophores have been synthesized.[7–10] The study of such systems and interpretation of the results in terms of modern electron transfer theory have contributed substantially to the understanding of photoinitiated electron transfer and the various factors affecting it. Nevertheless, the same factors that facilitate rapid photoinduced electron transfer in the dyads also facilitate charge recombination. Thus, dyad systems in general are unable to reproduce the long-lived charge separation (over a few hundred ps) characteristic of the photosynthetic reaction centre. As has been shown,[6,7] this limitation may be overcome by more complex models, for instance, by triad systems. The triads are capable of performing sequential, multistep photoinduced electron transfer, thus decreasing the probability of fast charge recombination and significantly prolonging the lifetime of the final charge-separated state. In continuation of our earlier work,[10–12] we present here the synthesis and characterization of the first phytochlorin-anthraquinone ether dyad and of the new triad molecules consisting of a covalently linked quinone moiety and two phorbin rings.

Results and discussion

Our synthetic strategy (Fig. 1) begins from 13^2-demethoxycarbonylchlorophyll *a* (pyroChl *a*, **1**) which was

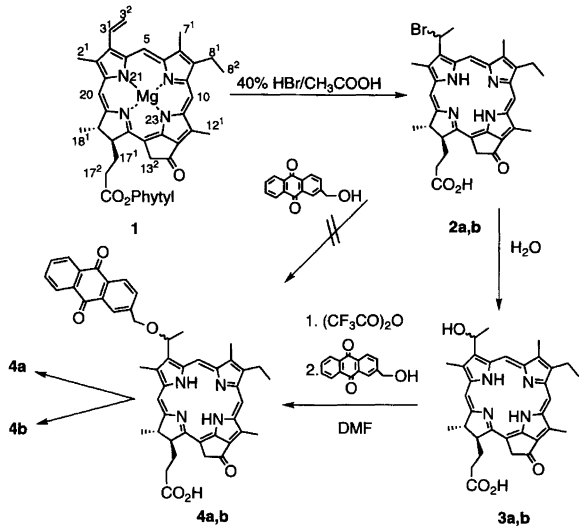

Fig. 1. Synthesis of $3^1(R,S)$-[(anthraquinon-2-yl)methoxy]-phytochlorin.

* To whom correspondence should be addressed.

obtained by heating a degassed pyridine solution of Chl *a* at 100 °C in a sealed tube[13]. In order to create a proper binding site for coupling with a quinone alcohol, we decided to α-brominate the vinyl group of the phorbin. This was achieved by treating pyroChl *a* with a 40% solution of HBr in CH_3COOH, stirred overnight, at room temperature, under an argon atmosphere. The α-bromination of the vinyl group, the complete hydrolysis of the phytyl chain and the demetallation of pyroChl *a* occurred simultaneously under the conditions used.

Attempts were made to couple directly the 3^1-bromophytochlorin thus obtained with 2-(hydroxymethyl)-anthraquinone to give the $3^1(R,S)$-[(anthraquinon-2-yl)methoxy]phytochlorin (**4a,b**). However, despite attempted optimization of the reaction conditions (solvents, temperature), only traces of the desired product (less than 2%) were obtained following this route. The main product of the reaction was found to be pyropheophorbide *a* (yield > 50%), implying that the elimination of HBr was the principal reaction under the conditions used. Since it was clear that 3^1-bromophytochlorin would not undergo the required reaction, we turned our attention to 3^1-hydroxyphytochlorin (**3a,b**) as a possible precursor for coupling with the quinone alcohol. The 3^1-hydroxyphytochlorin was obtained by hydrolysis of the 3^1-Br derivative (**2a,b**) with water for 1 h. After purification on a silica column using dichloromethane–methanol (7:1, v/v) as the eluent, the required derivatives **3a,b** were obtained in a 78% yield relative to pyroChl *a*.

A very convenient method has been reported[14] for the preparation of ether-bonded porphyrin dimers and oligomers from their hydroxy precursors by activating one of them as its trifluoroacetate. We used the same method in order to prepare phytochlorin–anthraquinone dyads (**4a,b**). The $3^1(R,S)$-hydroxyphytochlorin (**3a,b**) was first treated with trifluoroacetic anhydride (the reaction was complete after 1 h as judged by TLC) and the resulting trifluoroacetate was then condensed with the 2-(hydroxymethyl)anthraquinone in dry DMF at 20 °C, giving the dyads **4a,b** in a yield of 43%. The choice of solvent for the coupling reaction was found to be critical, as no amount of the desired product was detected by TLC when the reaction was carried out in CH_2Cl_2 or $CHCl_3$. This observation provides some clue as to the mechanism of the coupling reaction. The main competitive reaction to the coupling was found to be elimination, leading to the formation of pyropheophorbide *a*. The amount of pyropheophorbide *a* in the reaction mixture was substantially smaller in DMF compared with that in CH_2Cl_2 or $CHCl_3$. As has been reported,[15] polar aprotic solvents (such as DMF) promote the S_N2-type reactions at the expense of S_N1 or elimination. Thus, it is reasonable to propose, that the reaction of **3a,b** to **4a,b** occurs according to an S_N2 mechanism.

It was also found to be extremely important for the etherification which one of the two hydroxy groups [the hydroxy group of **3a,b** or that of 2-(hydroxymethyl)anthraquinone] was activated with trifluoroacetic anhydride. There was no coupling observed if the 3^1-hydroxyphytochlorin (**3a,b**) was reacted with the trifluoroacetic acid ester of 2-(hydroxymethyl)anthraquinone under the same conditions, implying that it is the secondary hydroxy function of **3a,b** that has to be activated in order to produce **4a,b**.

As expected, the dyad **4a,b** was composed of two diastereomers (ratio *ca.* 1:1) differing in the stereochemistry of the 3^1-chiral carbon atom. Ion-suppression HPLC was used for the separation of the diastereomers. The separation conditions were optimized using $C_8$and C_{18} reversed-phase sorbents. Finally, satisfactory separation (see the Experimental for the details) of the epimers was achieved, both on an analytical and on a semi-preparative scale, by means of a C_8 reversed-phase HPLC column (Merck LiChrospher 100 RP-8).

Because the conformational aspects are of great importance in the construction of new model compounds for photoinduced electron transfer, it was necessary to compare the solution conformations of **4a,b** with those of the pyropheophytin–anthraquinone dyad molecules already synthesized and studied by us.[10,11] In the work cited, prominent upfield shifts and the intramolecular NOE correlations observed in the ^1H NMR spectra of the pyropheophytin–anthraquinone dyads led us to the conclusion that, in $CDCl_3$ solutions, these dyads occur to a significant extent in folded conformations, in which the anthraquinone moiety is on the top of the phorbin ring. The intramolecular π–π interactions were supposed to be responsible for the folding. The ^1H NMR spectra of the phytochlorin–anthraquinone dyads (**4a,b**) in $CDCl_3$ solutions appeared, however, to be a superposition of their phorbin and quinone components without any significant upfield shifts of the proton resonances. Thus, folding similar to that observed in the solutions of the pyropheophytin–anthraquinone compounds, did not occur in the solutions of **4a,b**. We have interpreted this important observation as a consequence of a shorter and more rigid linkage between the phorbin and anthraquinone rings in dyads **4a,b**.

To accomplish the synthesis of triads **6a–d**, it was then necessary to modify the phytyl chain of pyroChl *a* (**1**) for the creation of a proper binding site for coupling with a carboxylic function of phytochlorin–anthraquinone **4a,b**. To achieve this goal, we considered the P4-hydroxypyropheophytin *a* (**5a,b**) to be a convenient derivative for the coupling reaction. As reported by us earlier,[10,16] compounds **5a,b** can be obtained from pyroChl *a* after 5 h of reflux in dioxane, using 4 equivalents of SeO_2. Removal of magnesium occurs simultaneously under the conditions described.[16]

Thus, after having obtained both building blocks, **4a,b** and **5a,b**, ready for construction of the desired triad molecules **6a–d**, the choice of the coupling reagent for the esterification became crucial. There are numerous mild methods suitable for the esterification of pheophorbides and related compounds with various types of alcohol, in the literature.[17] In our previous work,[10] we

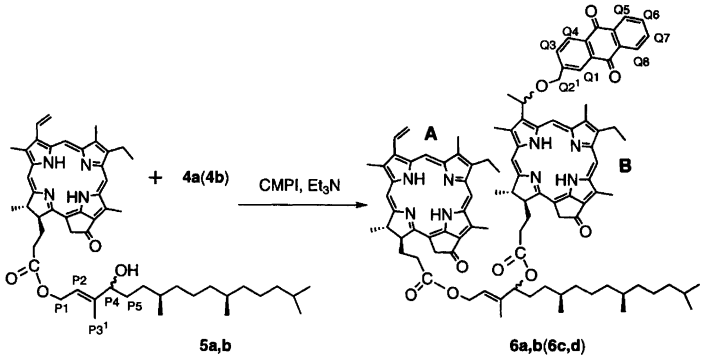

Fig. 2. Synthesis of the pyropheophytin–phytochlorin–anthraquinone triad molecules.

used *N,N'*-dicyclohexylcarbodiimide with a catalytic amount of 4-(dimethylamino)pyridine for esterification between the P4-hydroxy derivative **5a,b** and 9,10-anthraquinone-2-carboxylic acid. However, our attempts to use the same method afforded low yields, mainly as a result of significant formation of an unwanted *N*-acyldicyclohexylurea derivative of anthraquinone–phytochlorin **4a,b**. Attempts to apply the mixed anhydride method by using di-*tert*-butyl dicarbonate with a catalytic amount of 4-(dimethylamino)pyridine[8] were also unsatisfactory, as they afforded mainly the *tert*-butyl ester of **4a,b**.

To accomplish the synthesis of the target compounds, **6a–d**, 2-chloro-1-methylpyridinium iodide[18] (CMPI, Mukaiyama's reagent) in the presence of 2 equivalents of triethylamine, was used for the coupling. This method has been used successfully by Boxer and Bucks for the synthesis of their model for the photosynthetic reaction centre.[19] The syntheses of **6a–d** were performed in dry $CHCl_3$, at 40 °C, under argon. The P4-hydroxy derivative **5a,b** was coupled separately with the optically pure epimers, **4a** and **4b** (Fig. 2), resulting in two diastereomeric pairs, **6a,b** (60% yield) and **6c,d** (58%), respectively. The epimeric ratio was found from HPLC analysis to be ca. 1:1 for both diastereomeric pairs, assuming similar molar absorptivities for the components.

The separation of the diastereomers posed a difficult task. No separation between the epimers was achieved using C_8 or C_{18} reversed-phase HPLC columns. Partial separation of **6c** and **6d** epimers was observed using normal-phase silica HPLC columns with *n*-heptane–ethyl acetate mixtures [25–30% (v/v) of EtOAc] as the eluent. Unfortunately, the mobile phases based on *n*-heptane caused substantial broadening of the peaks. A likely reason for the broadening is the aggregation of compounds **6a–d** owing to their limited solubility in the mobile phase. To eliminate the aggregation by increasing the solubility, the tertiary mobile system, *n*-heptane–EtOAc–CH_2Cl_2, was used. The addition of 5% (v/v) of methylene chloride to the mobile phase (its final composition was 72:23:5 *n*-heptane–EtOAc–CH_2Cl_2, v/v/v), resulted in a significant improvement of the lineshape of the peaks and allowed us to resolve completely the diastereomeric mixture of **6c** and **6d**. The separation of the other pair of diastereomers, **6a** and **6b**, was more complicated. The satisfactory resolution between the epimers (Fig. 3) was achieved only applying the recycling HPLC technique. After the separation, the purity of the isomers **6a**, **6b**, **6c** and **6d** was shown to be >95% (HPLC and ^1H NMR).

The results of steady-state fluorescence measurements have shown significant quenching of fluorescence for all triad molecules obtained. For instance, only 3% of the fluorescence of P4-hydroxypyropheophytin (reference compound) in CH_3CN remained for the triad **6d** (similar results were obtained for the other epimers). The efficiency of the fluorescence quenching in the same solvent was at least three times that observed earlier for our

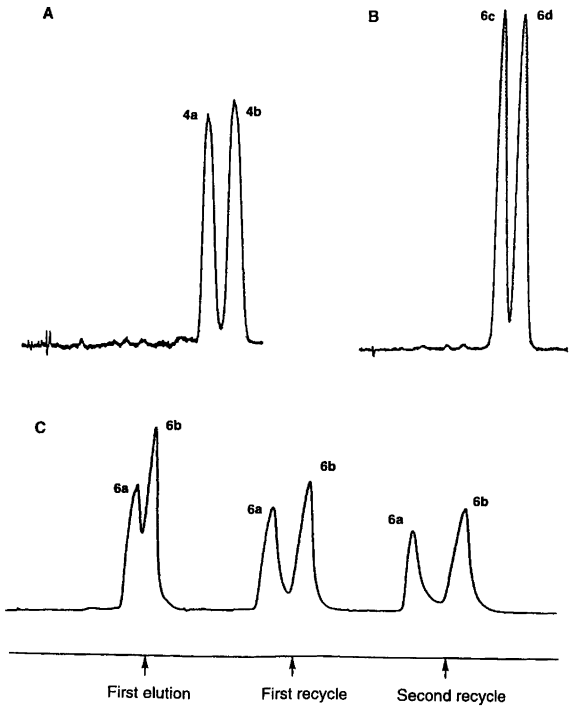

Fig. 3. HPLC separations of the mixtures of (A) diastereomers **4a** and **4b**; (B) diastereomers **6c** and **6d**; and (C) diastereomers **6a** and **6b**, with recycling. Chromatography conditions are as described in the Experimental.

pyropheophytin–anthraquinone model compounds.[10–12] This observation provides very attractive prospects for thorough photochemical investigations of the triads obtained.

Experimental

13^2-Demethoxycarbonylchlorophyll *a* (pyrochlorophyll *a*) (**1**) was prepared as described previously.[13] Silica gel 60 (230–400 mesh, ASTM, Merck, Darmstadt, Germany) was used for column chromatography. The HPLC experiments, both analytical and semi-preparative, were performed with a Waters (Milford, MA, USA) liquid chromatograph consisting of a Waters 600E multisolvent delivery system and a Waters 996 photodiode array detector. Components were monitored measuring the absorption at 410 nm. The NMR spectra were run on 200 MHz Varian GEMINI and 500 MHz Varian UNITY spectrometers. $CDCl_3$ (D% 99.5, Aldrich, Milwaukee, USA) was used as the solvent and tetramethylsilane as an internal reference. NMR ROESY experiments were performed in order to accomplish the full assignment of the ^{1}H NMR spectra of the triads **6a–d**. The conditions of the ROESY experiments were essentially the same as reported earlier.[20] Fast atom bombardment (FAB) mass spectra were measured with a Finnigan MAT 95 high resolution mass spectrometer equipped with an Ion Tech FAB gun (Teddington, England). The electronic absorption spectra were measured on a Varian Cary 5E UV–VIS–NIR spectrophotometer and the fluorescence spectra on a Shimadzu RF-5000 spectrofluorimeter. All solvents used were of analytical grade; chloroform and dichloromethane were distilled through a Vigreux column prior to use. 2-Hydroxymethylanthraquinone (97%) and 2-chloro-1-methylpyridinium iodide (CMPI, 97%) were purchased from Aldrich. Trifluoroacetic anhydride (>99%) was obtained from Fluka (Buchs, Switzerland).

3^1(R,S)-Hydroxyphytochlorin (**3a,b**). 460 mg of pyrochlorophyll *a* (**1**) were dissolved in 10 ml of 40% solution of HBr in CH_3COOH and then stirred at room temperature, overnight, under Ar, in the dark. To the solution of 3^1-bromophytochlorin (**2a,b**) thus obtained, without isolation, were added 2 ml of distilled water and the stirring was continued for 1 h. The reaction mixture was then poured into 100 ml of distilled water and extracted with 3 × 100 ml of $CHCl_3$. The combined extracts were washed with 100 ml of 5% sodium bicarbonate and then with 100 ml of water and dried on Na_2SO_4. After evaporation, the residue was chromatographed on a silica-gel column (230–400 mesh; height of the layer 280 mm; 300 × 45 mm ID column; elution with CH_2Cl_2–MeOH, 7:1, v/v) to yield 237 mg of **3a,b** (78%). The ^{1}H NMR spectrum of the methyl ester of **3a,b** (prepared for comparison purposes by treating 20 mg of **3a,b** with an excess of ethereal diazomethane) was virtually the same as reported earlier;[21] m.p. 247–249 °C (lit.[21] m.p. 245–247 °C).

3^1(R,S)-[(Anthraquinon-2-yl)methoxy]phytochlorin (**4a,b**). 50 mg of **3a,b** were dissolved in 2 ml of dry $CHCl_3$ and 1 ml of $(CF_3CO)_2O$ was added dropwise to the solution with stirring. The reaction mixture was protected from moisture and stirred for 1 h in dim light. The solvent and unchanged $(CF_3CO)_2O$ were then evaporated off on a rotary evaporator and the residue was dried on an oil-pump (at <100 Pa) for 1 h. A solution of 50 mg of 2-hydroxymethylanthraquinone in 5 ml of dry DMF was then added and reaction mixture was stirred overnight, under Ar, in the dark. The solution was diluted with 100 ml of CH_2Cl_2, washed with distilled water (3 × 100 ml) and dried (Na_2SO_4). After evaporation, the residue was chromatographed on a silica-gel column (230–400 mesh; height of the layer 280 mm; 300 × 45 mm ID column; elution with CH_2Cl_2–MeOH, 8:1, v/v) to give 30 mg of **4a,b** (43% yield), which was crystallized from chloroform–*n*-hexane. The mixture of the two diastereomers was separated by reversed-phase HPLC using a Merck LiChrospher 100 RP-8 column (250 × 10 mm ID), 5 μm. The mobile phase was 63% CH_3CN in H_2O, containing acetic acid 20 mM; flow rate, 6 ml min^{-1}.

Derivative **4a**: ^{1}H NMR (200 MHz, $CDCl_3$): δ_H 9.63 (s, 5-CH), 9.40 (s, 10-CH), 8.51 (s, 20-CH), 8.07–7.89 (m, Q1, Q4, Q5, Q8-CH_{arom}), 7.64–7.54 (m, Q3, Q6, Q7-CH_{arom}), 5.96 (q, ^3J=13 Hz, 3^1-CH), 5.20, 5.05 (AB spin system, $|^2J|$=19.7 Hz, 13^2-CH_2), 4.65 (s, $Q2^1$-CH_2), 4.47 (m, 18-CH), 4.25 (m, 17-CH), 3.65 (q, ^3J=7.7 Hz, 8^1-CH_2), 3.59 (s, 12^1-CH_3), 3.33 (s, 2^1-CH_3), 3.13 (s, 7^1-CH_3), 2.57 (m, 17^1-CH_2), 2.25 (m, 17^2-CH_2), 2.18 (d, ^3J=13 Hz, 3^2-CH_3), 1.78 (d, ^3J=7.3 Hz, 18^1-CH_3), 1.66 (t, ^3J=7.7 Hz, 8^2-CH_3), −0.08 (br s, 21-NH), −1.87 (br s, 23-NH); m.p. 198–200 °C (decomp.); UV–VIS ($CHCl_3$): λ_{max}/nm (ε/10^3 l mol^{-1} cm^{-1}): 257 (51.2), 320 (24.3), 409 (93.2), 506 (10.2), 536 (9.23), 606 (7.64), 664 (45.4); EI-MS (the corresponding methyl ester of **4a** was analysed, as the original carboxylic acid was non-volatile): *m/z* 786 (M^+); $C_{49}H_{46}N_4O_6$ requires 786.

Derivative **4b**: ^{1}H NMR (200 MHz, $CDCl_3$): δ_H 9.64 (s, 5-CH), 9.40 (s, 10-CH), 8.51 (s, 20-CH), 8.07–7.89 (m, Q1, Q4, Q5, Q8-CH_{arom}), 7.64–7.54 (m, Q3, Q6, Q7-CH_{arom}), 5.97 (q, ^3J=13 Hz, 3^1-CH), 5.21, 5.06 (AB spin system, $|^2J|$=19.7 Hz, 13^2-CH_2), 4.67 (s, $Q2^1$-CH_2), 4.47 (m, 18-CH), 4.25 (m, 17-CH), 3.65 (q, ^3J=7.7 Hz, 8^1-CH_2), 3.59 (s, 12^1-CH_3), 3.33 (s, 2^1-CH_3), 3.13 (s, 7^1-CH_3), 2.57 (m, 17^1-CH_2), 2.25 (m, 17^2-CH_2), 2.18 (d, ^3J=13 Hz, 3^2-CH_3), 1.80 (d, ^3J=7.3 Hz, 18^1-CH_3), 1.66 (t, ^3J=7.7 Hz, 8^2-CH_3), −0.08 (br s, 21-NH), −1.87 (br s, 23-NH); m.p. 198–200 °C (decomp.); UV–VIS ($CHCl_3$), λ_{max}/nm (ε/10^3 l mol^{-1} cm^{-1}): 257 (51.4), 320 (24.8), 409 (94.2), 506 (10.3), 536 (9.31), 606 (7.82), 664 (45.7); EI-MS (the corresponding methyl ester of **4b** was analysed, as the original carboxylic acid was non-volatile): *m/z* 786 (M^+); $C_{49}H_{46}N_4O_6$requires 786.

13^2-Demethoxycarbonyl-P4(R,S)-hydroxypheophytin a **5a,b**). Pyrochlorophyll *a* was regioselectively oxidized at the P4 position of its phytyl chain using 4 equivalents of

SeO_2 in dioxane with a yield of 32%, as reported by us earlier.[10,16] The spectroscopic properties (UV–VIS, ^1H NMR) of **5a,b** were consistent with those reported previously.[10]

13^2-Demethoxycarbonylpheophytin a-*P4(*R,S*)-yl-3^1(*R,S*)-[(anthraquinon-2-yl)methoxy]phytochlorin* (**6a–d**). 15 mg of 2-chloro-1-methylpyridinium iodide (CMPI) were added to a refluxing solution of 12 mg of **4a** and 25 mg of **5a,b** in 5 ml of dry $CHCl_3$ with stirring under Ar. After 10 min., 25 µl of Et_3N were added to the reaction mixture, refluxing was continued for 5 h and the progress of the reaction was monitored by TLC on silica [eluent $CHCl_3$–acetone 10:1 (v/v)]. The solvent was then evaporated off and the residue, dissolved in 5 ml of $CHCl_3$, was passed through the silica layer (70–230 mesh; height of the layer 60 mm; 70 × 45 mm ID glass sinter, elution with chloroform–acetone 1:1 v/v) and the effluent was evaporated to dryness. The residue was purified on a silica-gel column (230–400 mesh; height of the layer 330 mm; 350 × 25 mm ID column; elution with $CHCl_3$–acetone, 13:1 v/v) to give 15 mg of **6a,b** (60%). The mixture of the two diastereomers was separated by normal-phase high-performance LC using a Zorbax Sil column (250 × 9.4 mm ID), 5–6 µm (DuPont); mobile phase 72:23:5 *n*-heptane–EtOAc–CH_2Cl_2(v/v/v); flow rate 5.5 ml min^{-1}.

Derivative **6a** (rings A and B as presented in Fig. 2): ^1H NMR (500 MHz, $CDCl_3$): δ_H 9.61 (s, 5-CH, ring B), 9.46 (s, 10-CH, B), 9.38 (s, 10-CH, ring A), 9.20 (s, 5-CH, A), 8.58 (s, 20-CH, B), 8.32 (s, 20-CH, A), 8.01–7.89 (m, Q5, Q8-CH_{arom}), 7.86 (dd, $^3J_{cis}$ = 11.6 Hz, $^3J_{trans}$ = 17.8 Hz, 3^1-CH, A), 7.71 (m, Q1-CH), 7.62–7.56 (m, Q6, Q7, Q4-CH_{arom}), 7.22 (m, Q3-CH), 6.20 (dd, ^2J = 1.8 Hz, $^3J_{trans}$ = 17.8 Hz, 3^2-CH_2, H_{trans}, A), 6.10 (dd, ^2J = 1.8 Hz, $^3J_{cis}$ = 11.6 Hz, 3^2-CH_2, H_{cis}, A), 5.92 (q, ^3J = 13 Hz, 3^1-CH, B), 5.55 (m, P2-CH), 5.20, 5.01 (AB spin system, $|^2J|$ = 19.5 Hz, 13^2-CH_2, B), 5.10 (t, ^3J = 6.7 Hz, P4-CH), 4.95, 4.62 (AB spin system, $|^2J|$ = 19.5 Hz, 13^2-CH_2, A), 4.54 (m, P1-CH_2), 4.48 (m, 18-CH, B), 4.43, 4.37 (AB spin system, $|^2J|$ = 13 Hz, Q2^1-CH_2), 4.22 (m, 17-CH, B), 4.12 (m, 18-CH, A), 3.65 (s, 12^1-CH_3, B), 3.64 (q, ^3J = 7.8 Hz, 8^1-CH_2, B), 3.62 (q, ^3J = 7.8 Hz, 8^1-CH_2, A), 3.59 (s, 12^1-CH_3, A), 3.51 (m, 17-CH, A), 3.38 (s, 2^1-CH_3, B), 3.27 (s, 2^1-CH_3, A), 3.14 (s, 7^1-CH_3, A), 3.06 (s, 7^1-CH_3, B), 2.70–2.38 (m, $17^{1,2}$-CH_2, B), 2.38–1.98 (m, $17^{1,2}$-CH_2, A), 2.16 (d, ^3J = 13 Hz, 3^2-CH_3, B), 1.82 (d, ^3J = 7.2 Hz, 18^1-CH_3, B), 1.67 (t, ^3J = 7.8 Hz, 8^2-CH_3, A, B), 1.63 (s, P3^1-CH_3), 1.45 (m, P5-CH_2), 1.25 (d, ^3J = 7.2 Hz, 18^1-CH_3, A); the remaining phytyl chain resonances were virtually the same as for **5a,b**, 0.27, 0.15 (each br s, 21-NH A, B), −1.83, −1.99 (each s, 23-NH A, B); UV–VIS (THF), λ_{max}/nm ($\varepsilon/10^3$ l mol^{-1} cm^{-1}): 256 (31.5), 318 (23.7), 409 (103.1), 506 (10.6), 535 (9.27), 609 (7.89), 664 (46.3); FAB-MS: *m/z* 1584 ($M+H^+$); $C_{101}H_{114}N_8O_9$requires 1584.

Derivative **6b**: ^1H NMR (500 MHz, $CDCl_3$): δ_H 9.63 (s, 5-CH, B), 9.43 (s, 10-CH, B), 9.35 (s, 10-CH, A), 9.22 (s, 5-CH, A), 8.54 (s, 20-CH, B), 8.40 (s, 20-CH, A), 8.06–7.94 (m, Q5, Q8-CH_{arom}), 7.88 (dd, $^3J_{cis}$ = 11.6 Hz, $^3J_{trans}$ = 17.8 Hz, 3^1-CH, A), 7.86 (m, Q1-CH), 7.83 (m, Q4-CH), 7.66–7.58 (m, Q6, Q7-CH_{arom}), 7.44 (m, Q3-CH), 6.20 (dd, ^2J = 1.8 Hz, $^3J_{trans}$ = 17.8 Hz, 3^2-CH_2, H_{trans}, A), 6.10 (dd, ^2J = 1.8 Hz, $^3J_{cis}$ = 11.6 Hz, 3^2-CH_2, H_{cis}, A), 5.97 (q, ^3J = 13 Hz, 3^1-CH, B), 5.52 (m, P2-CH), 5.14, 4.96 (AB spin system, $|^2J|$ = 19.5 Hz, 13^2-CH_2, B), 5.10 (t, ^3J = 6.7 Hz, P4-CH), 5.06, 4.83 (AB spin system, $|^2J|$ = 19.5 Hz, 13^2-CH_2, A), 4.56 (m, Q2^1-CH_2), 4.54 (m, P1-CH_2), 4.44 (m, 18-CH, B), 4.28 (m, 18-CH, A), 4.16 (m, 17-CH, B), 3.88 (m, 17-CH, A), 3.64 (q, ^3J = 7.8 Hz, 8^1-CH_2, B), 3.60 (s, 12^1-CH_3, B), 3.59 (q, ^3J = 7.8 Hz, 8^1-CH_2, A), 3.57 (s, 12^1-CH_3, A), 3.37 (s, 2^1-CH_3, B), 3.29 (s, 2^1-CH_3, A), 3.13 (s, 7^1-CH_3, A), 3.12 (s, 7^1-CH_3, B), 2.66–2.44 (m, $17^{1,2}$-CH_2, B), 2.36–1.98 (m, $17^{1,2}$-CH_2, A), 2.19 (d, ^3J = 13 Hz, 3^2-CH_3, B), 1.79 (d, ^3J = 7.2 Hz, 18^1-CH_3, B), 1.67 (t, ^3J = 7.8 Hz, 8^2-CH_3, B), 1.65 (t, ^3J = 7.8 Hz, 8^2-CH_3, A), 1.60 (s, P3^1-CH_3), 1.52 (d, ^3J = 7.2 Hz, 18^1-CH_3, A), 1.43 (m, P5-CH_2); the remaining phytyl chain resonances were virtually the same as for **5a,b**, 0.22 (br s, 21-NH A, B), −1.86, −1.92 (each s, 23-NH A, B); UV–VIS (THF), λ_{max}/nm ($\varepsilon/10^3$ l mol^{-1} cm^{-1}): 256 (33.5), 318 (23.9), 409 (105.2), 506 (10.9), 535 (9.37), 609 (7.53), 664 (47.3); FAB-MS: *m/z* 1584 ($M+H^+$); $C_{101}H_{114}N_8O_9$ requires 1584.

The other pair of diastereomers (**6c,d**) were prepared from the **4b** epimer in 58% yield, in the same manner as for **6a,b**. The mixture of the two diastereomers was separated by normal-phase high-performance LC using a Zorbax Sil column (250 × 9.4 mm ID), 5–6 µm (DuPont); the mobile phase was 70:20:10 *n*-heptane–EtOAc–CH_2Cl_2 (v/v/v), multiple recycling with a flow rate of 7 ml min^{-1}. Fig. 3 shows the separation of **6c** and **6d** achieved upon recycling.

Derivative **6c**: ^1H NMR (500 MHz, $CDCl_3$): δ_H 9.67 (s, 5-CH, B), 9.44 (s, 10-CH, B), 9.37 (s, 10-CH, A), 9.25 (s, 5-CH, A), 8.51 (s, 20-CH, B), 8.42 (s, 20-CH, A), 8.08–7.94 (m, Q4, Q5, Q8-CH_{arom}), 7.93 (m, Q1-CH), 7.89 (dd, $^3J_{cis}$ = 11.6 Hz, $^3J_{trans}$ = 17.8 Hz, 3^1-CH, A), 7.64–7.56 (m, Q6, Q7-CH_{arom}), 7.53 (m, Q3-CH), 6.20 (dd, ^2J = 1.8 Hz, $^3J_{trans}$ = 17.8 Hz, 3^2-CH_2, H_{trans}, A), 6.10 (dd, ^2J = 1.8 Hz, $^3J_{cis}$ = 11.6 Hz, 3^2-CH_2, H_{cis}, A), 5.94 (q, ^3J = 13 Hz, 3^2-CH, B), 5.46 (m, P2-CH), 5.13, 4.95 (AB spin system, $|^2J|$ = 19.5 Hz, 13^2-CH_2, B), 5.11, 4.97 (AB spin system, $|^2J|$ = 19.5 Hz, 13^2-CH_2, A), 5.07 (t, ^3J = 6.7 Hz, P4-CH), 4.61, 4.57 (AB spin system, $|^2J|$ = 13 Hz, Q2^1-CH_2), 4.52 (m, P1-CH_2), 4.42 (m, 18-CH, B), 4.36 (m, 18-CH, A), 4.18–4.10 (m, 17-CH, A, B), 3.65 (q, ^3J = 7.8 Hz, 8^1-CH_2, B), 3.61 (q, ^3J = 7.8 Hz, 8^1-CH_2, A), 3.60 (s, 12^1-CH_3, B), 3.58 (s, 12^1-CH_3, A), 3.33 (s, 2^1-CH_3, B), 3.28 (s, 2^1-CH_3, A), 3.16 (s, 7^1-CH_3, A), 3.15 (s, 7^1-CH_3, B), 2.62–2.44 (m, $17^{1,2}$-CH_2, B), 2.36–1.98 (m, $17^{1,2}$-CH_2, A), 2.20 (d, ^3J = 13 Hz, 3^2-CH_3, B), 1.75 (d, ^3J = 7.2 Hz, 18^1-CH_3, B), 1.68 (t, ^3J = 7.8 Hz, 8^2-CH_3, A, B), 1.67 (d, ^3J = 7.2 Hz, 18^1-CH_3, A), 1.57 (s, P3^1-CH_3), 1.43 (m,

P5-CH_2); the remaining phytyl chain resonances were virtually the same as for **5a,b**, 0.28, 0.20 (each br s, 21-NH A, B), −1.86, −1.88 (each s, 23-NH A, B); UV–VIS (THF), λ_{max}/nm ($\varepsilon/10^3$ l mol^{-1} cm^{-1}): 256 (32.7), 318 (23.7), 409 (106.5), 506 (10.8), 535 (9.37), 609 (7.59), 664 (47.1); FAB-MS: *m/z* 1584 ($M+H^+$); $C_{101}H_{114}N_8O_9$ requires 1584.

Derivative **6d**: ^{1}H NMR (500 MHz, $CDCl_3$): δ_H 9.65 (s, 5-CH, B), 9.46 (s, 10-CH, B), 9.42 (s, 10-CH, A), 9.27 (s, 5-CH, A), 8.51 (s, 20-CH, B), 8.41 (s, 20-CH, A), 8.06–7.92 (m, Q4, Q5, Q8-CH_{arom}), 7.91 (m, Q1-CH), 7.89 (dd, $^3J_{cis}$ = 11.6 Hz, $^3J_{trans}$ = 17.8 Hz, 3^1-CH, A), 7.62–7.53 (m, Q6, Q7-CH_{arom}), 7.52 (m, Q3-CH), 6.21 (dd, ^2J = 1.8 Hz, $^3J_{trans}$ = 17.8 Hz, 3^2-CH_2, H_{trans}, A), 6.11 (dd, ^2J = 1.8 Hz, $^3J_{cis}$ = 11.6 Hz, 3^2-CH_2, H_{cis}, A), 5.92 (q, ^3J = 13 Hz, 3^1-CH, B), 5.48 (m, P2-CH), 5.17, 5.00 (AB spin system, $|^2J|$ = 19.5 Hz, 13^2-CH_2, B), 5.11, 4.91 (AB spin system, $|^2J|$ = 19.5 Hz, 13^2-CH_2, A), 5.05 (t, ^3J = 6.7 Hz, P4-CH), 4.60, 4.54 (AB spin system, $|^2J|$ = 13 Hz, Q2^1-CH_2), 4.52 (m, P1-CH_2), 4.44 (m, 18-CH, B), 4.32 (m, 18-CH, A), 4.20 (m, 17-CH, B), 4.04 (m, 17-CH, A), 3.67 (q, ^3J = 7.8 Hz, 8^1-CH_2, B), 3.65 (q, ^3J = 7.8 Hz, 8^1-CH_2, A), 3.63 (s, 12^1-CH_3, B), 3.60 (s, 12^1-CH_3, A), 3.33 (s, 2^1-CH_3, B), 3.29 (s, 2^1-CH_3, A), 3.19 (s, 7^1-CH_3, A), 3.15 (s, 7^1-CH_3, B), 2.62–2.38 (m, 17^{1,2}-CH_2, B), 2.38–1.98 (m, 17^{1,2}-CH_2, A), 2.19 (d, ^3J = 13 Hz, 3^2-CH_3, B), 1.75 (d, ^3J = 7.2 Hz, 18^1-CH_3, B), 1.68 (t, ^3J = 7.8 Hz, 8^2-CH_3, A, B), 1.61 (d, ^3J = 7.2 Hz, 18^1-CH_3, A), 1.57 (s, P3^1-CH_3), 1.44 (m, P5-CH_2); the remaining phytyl chain resonances were virtually the same as for **5a,b**, 0.28, 0.24 (each br s, 21-NH, A, B), −1.85, −1.88 (each s, 23-NH, A, B); UV–VIS (THF), λ_{max}/nm ($\varepsilon/10^{-3}$ l mol^{-1} cm^{-1}): 256 (32.2), 318 (23.9), 409 (103.2), 506 (10.7), 535 (9.35), 609 (7.61), 664 (46.6); FAB-MS: *m/z* 1584 ($M+H^+$); $C_{101}H_{114}N_8O_9$ requires 1584.

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References

1. Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. *J. Mol. Biol. 180* (1984) 385.
2. Deisenhofer, J., Epp, O., Miki, K., Huber, R. and Michel, H. *Nature 318* (1985) 618.
3. Deisenhofer, J. and Michel, H. *Science 245* (1989) 1463.
4. Allen, J. P., Feher, G., Yeates, T. O., Rees, D. C., Deisenhofer, J., Michel, H. and Huber, R. *Proc. Natl. Acad. Sci. USA 83* (1986) 8589.
5. Chang, C. H., Tiede, D., Tang, J., Smith, U., Norris, J. and Schiffer, M. *FEBS Lett. 205* (1986) 82.
6. Gust, D. and Moore, T. A. *Top. Curr. Chem. 159* (1991) 103 and references therein.
7. Wasielewski, M. R. *Chem. Rev. 92* (1992) 435 and the references therein.
8. Borovkov, V. V., Gribkov, A. A., Kozyrev, A. N., Brandis, A. S., Ishida, A. and Sakata, Y. *Bull. Chem. Soc. Jpn. 65* (1992) 1533.
9. Maruyama, K., Yamada, H. and Osuka, A. *Chem. Lett. 5* (1989) 833.
10. Tauber, A. Y., Kostiainen, R. K. and Hynninen, P. H. *Tetrahedron 50* (1994) 4723.
11. Tauber, A. Y., Helaja, J., Tkachenko, N. V., Lemmetyinen, H., Kilpeläinen, I. and Hynninen, P. H. In: Mathis, P., Ed., *Photosynthesis: from Light to Biosphere*; Kluwer, The Netherlands 1995, Vol. I, p. 815.
12. Tkachenko, N. V., Tauber, A. Y., Lemmetyinen, H. and Hynninen, P. H. *Thin Solid Films* (1996). *In press.*
13. Pennington, F. C., Strain, H. H., Svec, W. A. and Katz, J. J. *J. Am. Chem. Soc. 86* (1964) 1418.
14. Mironov, A. F., Nizhnik, A. N., Deruzhenko, I. V. and Bonnett, R. *Tetrahedron Lett. 31* (1990) 6409.
15. Cooper, K. A., Dhar, M. L., Hughes, E. D., Ingold, C. K., MacNulty, B. J. and Woolf, L. I. *J. Chem. Soc.* (1948) 2043.
16. Tauber, A. Yu. and Hynninen, P. H. *Tetrahedron Lett. 34* (1993) 2979.
17. For the leading reference see Wasielewski, M. R. and Svec, W. A. *J. Org. Chem. 45* (1980) 1969.
18. Saigo, K., Usui, M., Kikuchi, K., Shimada, E. and Mukaiyama, T. *Bull. Chem. Soc. Jpn. 50* (1977) 1863.
19. Boxer, S. G. and Bucks, R. R. *J. Am. Chem. Soc. 101* (1979) 1883.
20. Helaja, J., Hyvärinen, K., Heikkinen, S., Kilpeläinen, I. and Hynninen P. H. *J. Mol. Struct. 354* (1995) 71.
21. Smith, K. M., Bisset, G. M. F. and Bushell, M. J. *J. Org. Chem. 45* (1980) 2218.

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