Enzyme-Catalyzed Organic Syntheses: Transesterification Reactions of Chlorophyll *a*, Bacteriochlorophyll *a*, and Derivatives with Chlorophyllase

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Abstract: We have used the green plant enzyme chlorophyllase (EC 3.1.1.14, chlorophyll chlorophyllido-hydrolase) for the synthesis of a variety of primary alcohol and diol esters of chlorophyll *a*, bacteriochlorophyll *a*, and pyrobacteriochlorophyll *a*. Green plant chlorophyllase accepts a much larger range of alcohol and chlorophyll substrates than had previously been realized. Thus, chlorophyllide and bacteriochlorophyllide esters of primary alcohols such as retinol and the detergent Triton X-100 and of dihydric alcohols such as ethylene glycol, butanediol, or 2-hydroxyethyl disulfide can readily be obtained by enzyme-assisted transesterification. The diol chlorophyllide esters are valuable intermediates for the synthesis of reaction center "special pair" models. Chlorophyllase-assisted reactions can be carried out in media containing up to 95% of organic solvents without the concomitant side reactions that important chlorophyll functional groups readily undergo even under mild conditions in conventional chemical synthetic procedures. In competitive chlorophyllase-catalyzed transesterification reactions, long-chain alcohols such as farnesol and retinol vs simple aliphatic alcohols and diols, the enzyme shows a definite preference for the long-chain alcohol.

Much of recent chlorophyll chemistry has focused on the synthesis of models of photosynthetic reaction center chlorophylls.[1-3] Synthetic work with the chlorophylls has been plagued by the many side reactions that the chlorophylls readily undergo. Loss of the central magnesium atom,[4] oxidation by mild oxidants,[5] light-induced rearrangements,[6] C-10 epimerization,[7] allomerization,[8] and decarbomethoxylation limit the applicability of many synthetic procedures. As a result, porphyrins and the more stable pyroderivatives of chlorophylls frequently replace intact chlorophylls for the synthesis of model systems.[9,10] Difficulties in the reinsertion of the magnesium atom, particularly in the case of bacteriochlorophylls *a*[11] *b*, and *g*, further complicate the problem. Synthetic procedures (based on the application of the Wittig reaction to intact chlorophylls possessing the formyl group) that avoid some of these problems have recently been introduced.[12,13] We have turned to enzymatic reactions to ameliorate some of the conventional synthesis problems in chlorophyll chemistry. As the chlorophylls are insoluble in aqueous solution, an enzymatic approach requires enzymes active in organic solvents. There is growing interest in the application of such enzymes to chemical synthesis.[14-16]

Table I. Solvent Effects in the Reaction of **1** and **4** with Ethyl Alcohol (A) and Ethylene Glycol (B)[a]

compd	H_2O	acetone	THF	A	B	product ratio[b]	reactn time, h	reactn progress,[c] %
1	16	67		17		1	4.5	60
1	16		67	17		0.2	20	15
4	22	44			32	6	3	99
4	22		44		32	6	3	99

[a] 0.5 μmol of **1** or **4** per 100 mg of enzyme. All solutions given in percent (v/v). Pyridine, 2%, present in all reactions. Esters **1b** and **10** are formed. [b] Measured at the time of reaction termination as a transesterification/hydrolysis ratio. [c] Amount of **1** or **4** reacted.

The enzyme chlorophyllase (chlorophyll chlorophyllido-hydrolase, EC 3.1.1.14), discovered by Willstätter and Stoll[17] in green plants, catalyzes the esterification (or hydrolysis)[18,19] of the phytyl, geranylgeranyl, or farnesyl moieties at the propionic acid side chain of the chlorophyll macrocycle.[20] Chlorophyllase may well have been the first enzyme discovered to retain its enzymatic activity in organic solvents. Willstätter and Stoll used it in 92% alcohol solution for the first synthesis of methyl and ethyl chlorophyllides *a* and *b*.[21] The hydrolytic activity of the enzyme has received more attention from subsequent investigators than its capacity to catalyze transesterification reactions.[22,23]

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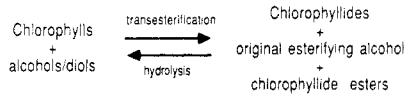
Table II. Enzymatic Transesterification of Chlorophylls with Alcohols and Diols

compd	yield, %	enzyme/ substrate ratio[a]	water, % (v/v)	organic/ alcohol ratio[b]	alcohol or diol, % (v/v)	reactn time, h	transesterification/ hydrolysis ratio[c]
5	28	120	17.0	2.7	22.7	17.5	0.8
6	51	116	18.5	2.7	37.0	4.0	1.0
7	41	200	7.4	1.5	37.0	24.0	7.0
8	34	125	16.9	13.0	5.9	18.0	3.3
9	35	100	17.1	35.0	2.3	22.0	0.8
10	76	100	11.1	2.0	44.4	24.0	13.3
	11	125	17.0	13.0	5.0	18.0	0.2

[a] Milligrams of acetone powder chlorophyllase per milligram of pigment. [b] Ratio of other organic solvents to alcohol or diol. [c] Measured at the time of reaction termination.

We have found that chlorophyll *a* (**1**), pyrochlorophyll *a* (**2**), pyropheophorbide *a* (**2a**), bacteriochlorophyll *a* (**3**), and pyrobacteriochlorophyll *a* (**4**) can be transesterified with diols and with a much broader range of alcohols than had previously been suspected. Thus, chlorophyllide esters of the primary alcohols, diols, retinol, and the detergent Triton X-100[24] can readily be obtained by chlorophyllase-assisted transesterification.

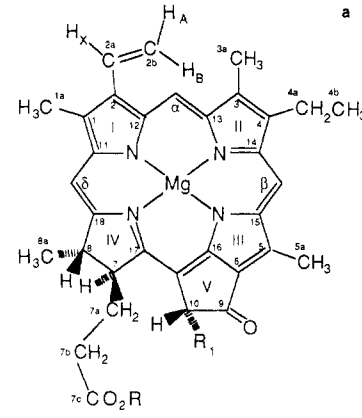


Results and Discussion

We describe here the general features of the preparation of chlorophyllide *a*, pyrochlorophyllide *a*, pyropheophorbide *a*, and pyrobacteriochlorophyllide *a* esters of aliphatic alcohols and diols on a milligram scale by chlorophyllase-catalyzed transesterification reactions (Figure 1a,b). Chlorophyllase[27,35] (see Experimental Section) retains its enzymatic activity for chlorophyll hydrolysis in aqueous acetone. Transesterification occurs when a primary alcohol or diol is present in the reaction solution. Hydrolysis competes with transesterification as observed with other esterases,[28,29] and a large molar excess of alcohol or diol is required to achieve high yields of chlorophyllide esters. The byproduct chlorophyllide formed by hydrolysis is a valuable starting material for the synthesis of chlorophyll derivatives.[22a] Removal of the solid "acetone powder" particles by filtration always terminates the reaction. Thus, the chlorophyllase preparation can be regarded as an immobilized enzyme on a natural support.

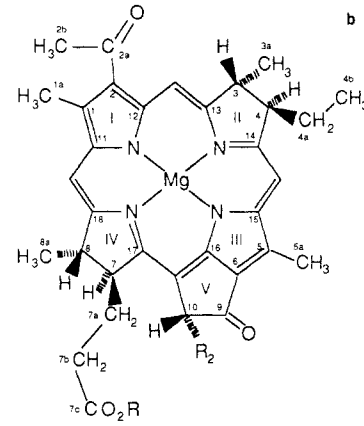
To maintain both substrate solubility (high organic solvent level) and enzyme activity (sufficient water content), the water content of the reaction medium was kept at 10–30% (v/v). Pyridine (1–2%, v/v) increases the solubility of chlorophylls in the reaction media and prevents loss of magnesium. In contrast to some lipases,[30] our chlorophyllase preparations are almost inactive in pure pyridine. Triton X-100 (0.25–0.75%, v/v) facilitates solubilization of the enzyme, but we do not recommend the routine use of this detergent.[24] Triton X-100 is widely used in the extraction of membrane proteins and chlorophyllase in particular.[25] This primary alcohol undergoes transesterification with chlorophylls, accounting for the alleged inhibitory action of Triton X-100[24,26] on chlorophyllase activity. Klibanov reports that another commonly used detergent, *n*-octyl β-D-glucopyranoside, undergoes acylation in organic media in lipase-catalyzed reactions.[30]

To explore the synthetic applications of chlorophyllase, various experimental conditions were examined on an analytical scale. The effect of water-miscible solvents on the product distribution and reaction progress was examined (Table I). Transesterification



1: R = phytyl, $R_1 = CO_2CH_3$
1a: R = H, $R_1 = CO_2CH_3$
1b: R = ethyl, $R_1 = CO_2CH_3$
2: R = phytyl, R_1 = H
2a*: R = phytyl, R_1 = H
5: R = $O(CH_2)_4OH$, $R_1 = CO_2CH_3$
6: R = $O(CH_2)_2OH$, R_1 = H
7*: R = $O(CH_2)_2S_2(CH_2)_2OH$, R_1 = H

*magnesium atom is replaced by 2H



3: R = phytyl, $R_2 = CO_2CH_3$
4: R = phytyl, R_2 = H
4a: R = H, R_2 = H
8: R = farnesyl, R_2 = H
9: R = retinyl, R_2 = H
10: R = $O(CH_2)_2OH$, R_2 = H

Figure 1. (a) Chlorophyll *a*, chlorophyllide *a*, and pyrochlorophyllide *a* esters formed in chlorophyllase-catalyzed reactions. (b) Bacteriochlorophyll *a* and pyrobacteriochlorophyllide *a* esters formed in chlorophyllase-catalyzed reactions.

of **1** with ethanol proceeds at quite different rates in media containing acetone of THF. In the acetone/water mixture, reaction with ethanol is faster and equal amounts of chlorophyllide *a* (**1a**) and ethyl chlorophyllide *a* (**1b**) are formed. When acetone is replaced by THF, 85% of **1** remains unreacted after 20 h. However, transesterification/hydrolysis of **4** with ethylene glycol proceeds smoothly in both acetone and THF. The reaction is essentially complete in 3 h and yields predominantly the trans-

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esterification product. Substitution of formamide for acetone (15–35%) had no noticeable effect.

Preparative-scale reactions of **1**, **2**, and **4** with diols and alcohols are summarized in Table II. Increasing the substrate/enzyme ratio results in longer reaction times and an increase in the hydrolysis/transesterification ratio. The amount of hydrolysis decreases as the concentration of water is decreased. Similar behavior was noticed in lipase-catalyzed hydrolysis.[31] When the amount of ester, as determined by analytical HPLC, reached its peak, the reaction was terminated by addition of acetone and removal of chlorophyllase by filtration. The yields of chlorophyllide esters formed in reactions of **2** and **4** with diols decreased after 90% of the chlorophyll was converted because of hydrolysis of the newly formed chlorophyllide esters. This effect was not observed when farnesol and retinol were used as substrates.

Isolation of the products from the crude reaction mixture containing a large excess of diols or alcohols is possible because the chlorophylls form filterable colloidal precipitates upon addition of a large excess of water. The chlorophyllides and the newly formed esters were isolated chromatographically. When reactions were run for longer than 24 h, a gradual loss of magnesium took place, even in the presence of pyridine.

Yields of pyrobacteriochlorophyllide esters **8**–**10**, formed in reactions of **4** with farnesol, retinol, and ethylene glycol, respectively, show that both farnesol and retinol can be used at lower concentrations (v/v) than diols in transesterification reactions. Ethylene glycol (5%, v/v), under conditions used in the preparation of **8**, produced **10** in only 11% yield, and hydrolysis was the dominant reaction. The transesterification/hydrolysis ratio in the formation of **8** and **9** was unchanged at a lower retinol and farnesol concentration of 0.4% (v/v). Inhibition of chlorophyllase hydrolytic activity by phytol has been reported previously.[32] One feature common to phytol, retinol, and farnesol is that they all possess an allylic alcohol function. Possible mechanisms of inhibition of chlorophyllase hydrolysis by phytol, farnesol, and retinol will be discussed elsewhere.

Conclusion

Chlorophyllase acetone powder catalyzes transesterification of chlorophylls and bacteriochlorophylls with primary alcohols and diols, without the loss of the central magnesium atom. The enzymatic approach offers a new route for the synthesis of photosynthetic models and considerably simplifies their preparation.[33,34] Chlorophyllase is readily available as an acetone powder (a naturally supported immobilized enzyme system), is easy to store, and is active in a variety of organic solvent/water systems. We believe it will find increasing use in chlorophyll chemistry.

Experimental Section

Materials. Chlorophyllase acetone powder was prepared from chloroplasts of *Ailanthus altissima* leaves[27] extracted according to the method of Tanaka,[35] modified by the addition of 0.2% pyridine (v/v) during chloroplast isolation and an additional extraction of the chloroplasts with acetone at a final concentration of 95%. Chlorophyllase acetone powder, vacuum dried, can be stored at −24 °C for several months with a loss of hydrolytic activity of less than 10%. Reagent-grade chemicals were used without further purification. Solvents were of HPLC grade. DEAE-Sepharose CL-6B was from Pharmacia. Chlorophyll *a*, pyrochlorophyll *a*, and pyropheophorbide *a* were prepared as previously described.[36]

Chromatography. HPLC analytical determinations were carried out with a dual-pump Beckman 110A system equipped with a Hewlett-Packard (HP) 8451A detector. The LC SURVEY (HP) program was used for data reduction. Development of the chromatogram on an Ultrasphere ODS, 5-μm (4.6 mm × 25 cm) column was followed simultaneously at 11 different wavelengths, and UV–vis spectra from 350 to 550 nm or 600 to 800 nm were recorded every 2 s. The relative amounts of chlorophyll and their derivatives were quantified by integration. The extinction coefficients, in a solvent system in which aggregation could be excluded, were assumed to be the same for each group of chlorophyll derivatives that differed only at the propionic acid chain. Preparative-scale separations were carried out on a Du Pont Zorbax column (21.2 mm × 25 cm) with an Altex Model 156 refractive index detector. The mobile phase consisted of acetone/ethanol/water (72/20/8, v/v), unless otherwise indicated.

General Methods. ^{1}H NMR spectra were recorded on Brucker AM (300-MHz) and Nicolet NT (200-MHz) spectrometers. ^{1}H NMR samples were dried by azeotropic removal of water, first with benzene, then $CDCl_3$, and, finally, CCl_4. Samples were prepared in $CDCl_3$ (TMS) and acetone-d_6 and diluted with up to 80% pyridine-d_5 when required to prevent aggregation. Average molecular weights of products were determined by ^{252}Cf PDMS.[37]

Chlorophyllase Assay. The hydrolytic activity of chlorophyllase acetone powder was determined by HPLC assay, with **1** as substrate. Acetone powder (66 mg), ascorbic acid (20 mg), and 0.4 mL of water were gently agitated and sonicated for 30 s; 0.5 mL of acetone was then added. Reaction was initiated by addition of 1 mg of **1** (1.12 μmol) in 0.5 mL of acetone. Incubation was carried out at 22 °C in the dark under N_2. The change in concentration of **1** and/or chlorophyllide *a* (**1a**) was used to follow the progress of the hydrolysis reaction. Aliquots of 5 μL taken every 5 min, diluted to 0.130 mL with acetone, and 10-μL samples were analyzed by HPLC. Activity of the chlorophyllase [defined here as micromoles of hydrolyzed **1** min^{-1} (mg of acetone powder)$^{-1}$] was 3.8×10^{-4} (at 30 min). Under these assay conditions, 96% of **1** is hydrolyzed to **1a** by 70 min.

Bacteriochlorophyll *a* (3). Freeze-dried *Rhodobacter sphaeroides* (1 g) was extracted with 3 × 3 mL of acetone to remove carotenes. The solid residue was extracted with 3 × 3 mL of methanol. The dark green solution was filtered through an acetone-washed Sep-Pak C-18 filter (Waters Associates) and evaporated. The residue was solubilized in 1.5 mL of acetone/ethanol/H_2O (72/20/8, v/v) and filtered in a LID-X/ORG.45 solid–liquid separator. Pigments were separated on the Zorbax column. Product **3**: retention time 27 min, flow rate 6 mL/min; 9 mg.

Pyrobacteriochlorophyll *a* (4). Compound **3** (100 mg) was refluxed in 20 mL of distilled collidine/pyridine (4/1, v/v) for 2 h. After evaporation (0.5 mmHg), the residue was solubilized in acetone, filtered through a LID-X filter, and purified on a Zorbax column in fractions corresponding to 20 mg of the crude product. Each separation (flow rate 7 mL/min, retention time 30 min) gave 12 mg of **4**, 64% yield.

Enzymatic Transesterification—General Procedure. Pigments dissolved in acetone and/or pyridine were added to chlorophyllase acetone powder (enzyme) suspended in a mixture of water, acetone, and alcohol/diol. Reaction progress was monitored by analytical HPLC. The enzyme was removed by filtration. The filtrate was diluted with an amount of cold water sufficient to precipitate the products. The green suspension was filtered through a (1 cm × 3 cm) 30–40-μm, C-18 glass column, and products remained absorbed on the top layer. The column was washed with 50 mL of water under suction. Chlorophylls were removed with 25 mL of acetone followed by 25 mL of 1/1 acetone/ethanol mixture and passed through a DEAE-Sepharose CL-6B column (2.5 cm × 15 cm) with acetone. Chlorophyllides were retained on the column. After concentration, chlorophyllide esters were isolated on the Zorbax column.

Enzymatic Synthesis of 4-Hydroxybutyl Chlorophyllide *a* (5). Chlorophyll *a* (**1**), 25 mg in 5 mL of acetone, was added to 3 g of enzyme suspended in 6 mL of water, 8 mL of 1,4-butanediol, 16 mL of acetone, 0.08 g of ascorbic acid, and 0.25 mL of pyridine. After 17.5 h, 49% of **1a** and 38% of **5** were formed with 13% of **1** unreacted. Chromatography (retention time 22 min, flow rate 2.5 mL/min) gave **5**: 6.1 mg, 26% yield; UV–vis (acetone) 432 nm (vs), 618 (w), 662 (vs) ^{1}H NMR [(200 MHz, $CDCl_3$/acetone-d_6 (1/1, v/v)] δ 9.58, 9.29 (each s, α- and β-meso H), 8.40 (s, δ-meso H), 8.02 (X of ABX, 2a-H_X), 6.19 (s, C-10-H), 6.18 (B of ABX, 2b-H_B), 6.02 (A of ABX, 2b-H_A), 4.48 (dq, 8-H), 4.15 (m, 7-H), 3.83 (s, 10b-CH_3), 3.78 (m, $COOCH_2$), 3.75 (m, 4a-CH_2), 3.61 (s, 5a-CH_3), 3.32 (s, 1a-CH_3), 3.27 (s, 3a-CH_3), 2.99 (t, CH_2O), 1.74 (t, 4b-CH_3), 1.68 (d, 8a-CH_3), 1.29 (m, CH_2CH_2); ^{252}Cf PDMS, calcd average $[M]^+$ 687.08, $[M - (CH_2)_4 - 2H]^+$ 628.97; observed $[M]^+$ 687.08, $[M - (CH_2)_4 - 2H]^+$ 628.11.

Enzymatic Synthesis of 2-Hydroxyethyl Pyrochlorophyllide *a* (6). Pyrochlorophyll *a* (**2**), 11 mg in 1 mL of acetone/pyridine (1/1, v/v), was added to 1.28 g of an enzyme in 5 mL of water, 50 mg of ascorbic acid, 0.05 mL of Triton X-100, 10 mL of acetone, 1 mL of formamide,

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and 10 mL of ethylene glycol. After 4 h, chromatography (retention time 16 min, flow rate 3 mL/min) gave **6**: 4 mg, 51% yield; UV–vis (acetone) 362 nm (s), 432 (vs), 662 (s2); ^{1}H NMR [300 MHz, $CDCl_3$/acetone-d_6/pyridine-d_5 (1/1/1, v/v)] δ 9.76, 9.44 (each s, α- and β-meso H), 8.14 (X of ABX, 2a-H_X), 6.24 (B of ABX, dd, J_{bb} = 1.1 Hz, J_{bx} = 18 Hz, 2b_B-H), 5.98 (A of ABX, dd, J_{AB} = 1.1 Hz, J_{AX} = 11.5 Hz, 2b_A-H), 5.22, 5.05 (ABq, J = 19.8 Hz, 10-CH_2), 4.46 (q, 8-H), 4.27 (d, 7-H), 4.12 (t, J = 4.7 Hz, $COOCH_2$), 3.78 (q, 4a-CH_2), 3.70 (s, 5-CH_3), 3.66 (t, OCH_2), 3.37 (s, 1-CH_3), 3.24 (s, 3-CH_3), 1.70 (t, J = 7.5 Hz, 4b-CH_3), 1.62 (d, 8-CH_3). Average molecular weight was determined for the demagnesiated derivative of **6**, 2-hydroxyethyl pyropheophorbide *a*: ^{252}Cf PDMS, calcd average $[M]^+$ 578.70, [M – $(CH_2)_2OH$], 533.64; observed $[M]^+$ 577.96, $[M - (CH_2)_2OH]^+$ 535.49.

Enzymatic Synthesis of 2-Hydroxyethyl Disulfide Pyropheophorbide *a* (7). Pyrochlorphyll *a* (**2**), 5 mg in 5 mL of acetone, was added to 1 g of enzyme in 2 mL of water, 10 mL of acetone, and 10 mL of 2-hydroxyethyl disulfide. After 24 h, the filtrate was concentrated by evaporation (0.1 mmHg) at 50 °C. The residue was extracted with 3 × 25 mL of ethyl ether, washed with 5 mL of 5% HCl and 4 × 10 mL of water, and concentrated. Analysis of the crude product [(ODS, 5μm, 10 mm × 250 mm, flow rate 3 mL/min, $CH_3OH/CH_3CN/THF$ (5/3.5/1.5, v/v), detection at 415 nm)] showed the presence of three major species: pyropheophorbide *a* (10%, retention time 3.97 min), **7** (70%, room temperature 6.70 min), and unreacted **2a** (6%, retention time 44.29 min). HPLC separation on a 5-μm SiO_2 column (100 mm × 10 mm) with $CHCl_3/CH_3COCH_3$ (9/1, v/v) as eluent, gave 90% enriched **7** further purified on a Zorbax column: [$CH_3OH/CH_3CN/THF$ (75/14.3/10.7, v/v), retention time 13 min, flow rate 6 mL/min] 1.7 mg, 41% yield; visible spectrum (acetone) 410 nm (vs), 506 (w), 534 (w), 608 (w), 664 (s); ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.58, 9.45 (each s, α- and β-meso H), 8.63 (s, δ-meso H), 8.00 (X of ABX, 2a-H_X), 6.29 (B of ABX, 2b-H_B), 6.19 (A of ABX, 2b-H_A), 5.22 (ABq, 10-CH_2), 4.52 (dq, 8-H), 4.33 (m, 7-H), 4.25 (t, $COOCH_2$), 3.75 (t, CH_2O), 3.72 (q, 4a-CH_2), 3.68 (s, 1a-CH_3), 3.42 (s, 3a-CH_3), 3.25 (s, 5a-CH_3), 2.76 (t, SCH_2), 2.75 (t, s-CH_2), 2.79–2.67, 2.59–2.52, 2.37–2.17 (each m, 7ab-CH_2CH_2), 1.83 (d, 8-CH_3), 1.69 (t, 4b-CH_3); ^{252}Cf PDMS, calcd average $[M]^+$ 670.81, $[M - O(CH_2)_2S]^+$ 594.76, $[M - (CH_2)_2 - S - S - (CH_2)_2OH]^+$ 533.65; observed, $[M]^+$ 671.32, $[M - O(CH_2)_2S]^+$ 595.34, $[M - (CH_2)_2 - S - S - (CH_2)_2 - OH]^+$ 534.39.

Enzymatic Synthesis of Farnesyl Pyrobacteriochlorophyllide *a* (8). Pyrobacteriochlorophyll *a* (**4**), 8 mg in 2 mL of acetone, was added to 1 g of enzyme in 2 mL of water, 0.03 g of ascorbic acid, 0.7 mL of farnesol (mixture of isomers), 7 mL of acetone, and 0.1 mL of pyridine. After 18 h, 15% of **4a** and 49% of **8** were formed with 36% of **4** unreacted. Fraction of 25–34 min, flow rate 2.5 mL/min, was concentrated under reduced pressure, until separation of phases occurred. Water was removed, and 0.75 mL of ethyl alcohol was added to the residue. Slow addition of 0.5 mL of water resulted in formation of a green precipitate. After centrifugation, the supernatant was removed and precipitation was repeated. Product **8**: (retention time 35 min, flow rate 3 mL/min) 2.5 mg, 34% yield; UV–vis (acetone) 354 nm (vs), 392 (s), 582 (w), 770 (vs); ^{1}H NMR (300 MHz, $CDCl_3$/acetone-d_6 (1/2, v/v) δ 9.01, 8.51 (each s, α- and β-meso H), 8.46 (s, δ-meso H), 5.29 (t, f-2, H), 4.93 (dd, 10-CH_2), 4.53 (d, f-1, CH_2), 3.58 (s, 1a-CH_3), 3.53 (s, 5a-CH_3), 3.04 (s, 2b-CH_3), 2.66–2.51, 2.43–2.30, 2.28–2.13 (each m, 7ab-CH_2CH_2), 1.62 (d, 8a-CH_3); ^{252}Cf PDMS, calcd average $[M]^+$ 778.31, [M – farnesyl]$^+$ 572.95, [farnesyl]$^+$ 205.36; observed $[M]^+$ 777.19, [M – farnesyl]$^+$ 574.41.

Enzymatic Synthesis of Retinyl Pyrobacteriochlorophyllide *a* (9). Pyrobacteriochlorophyll *a* (**4**), 10 mg in 1 mL of acetone/pyridine (1/1, v/v), was added to 1 g of enzyme in 3 mL of water, 1 mL of formamide, 11 mL of acetone, 0.4 g of retinol, 0.04 mL of Triton X-100, and 0.3 g of ascorbic acid. After 22 h, 49% of **4a** and 41% of **9** were formed with 10% of **4** unreacted. Product **9**: (retention time 10 min, flow rate 5 mL/min) 3.25 mg, 35% yield; UV–vis (ethyl ether) 280 nm (vs), 354 (vs), 390 (s), 574 (w), 770 (vs); ^{1}H NMR (300 MHz, $CDCl_3$/acetone-d_6) 9.05, 8.58 (each s, α- and β-meso H), 8.49 (s, δ-meso H), 6.63 (dd, r-5, H), 6.23 (d, r-4, H), 6.16 (d, r-9, H), 6.09 (d, r-8, H), 5,50 (t, r-2, CH_2), 5.05, 4.99 (ABq, 10-CH_2), 4.65 (d, r-1, CH_2), 4.25 (m, 8-H), 4.16 (m, 3-H), 4.08 (m, 4-H), 4.00 (m, 7-H), 3.54 (s, 1a-CH_3), 3.48 (s, 5a-CH_3), 3.06 (s, 26-CH_3), 2.03 (s, r-7a-CH_3), 1.92 (s, r-3a-CH_3), 1.70 (s, r-15a-CH_3), 1.02 (s, r-11a-CH_3 and r-11b-CH_3); ^{252}Cf PDMS, calcd average $[M]^+$ 841.39, [M – retinyl]$^+$ 571.95, [retinyl]$^+$ 269.44; observed, $[M]^+$ 844.77, [M – retinyl]$^+$ 573.98, [retinyl]$^+$ 269.51.

Enzymatic Synthesis of 2-Hydroxyethyl Pyrobacteriochlorophyllide *a* (10). Pyrobacteriochlorophyll *a* (**4**), 14 mg in 1 mL of acetone/pyridine (1/1), was added to 1.4 g of enzyme in 3 mL of water, 0.05 mL of Triton X-100, 0.04 g of ascorbic acid, 10 mL of acetone, 1 mL of formamide, and 12 mL of ethylene glycol. After 2 h 10 min 14%, 4 h 5 min 26%, 5 h 25 min 32%, and 24 h 93% of **10** was formed. Product **10**: (retention time 18 min, flow rate 4 mL/min) 7 mg, 76% yield; UV–vis (ethyl ether) 358 nm (s), 392 (s), 576 (w), and 770 (vs); ^{1}H NMR [200 MHz, $CDCl_3$/acetone-d_6/pyridine-d_5 (1/1/1, v/v)] δ 9.18, 8.57 (each s, α- and β-meso H), 8.46 (s, δ-meso H), 5.07, 4.98 (ABq, J = 19.9 Hz, 10-CH_2), 4.16 (t, $COOCH_2$), 3.72 (t, OCH_2), 3.56 (s, 1a-CH_3), 3.50 (s, 5a-CH_3), 3.07 (s, 2b-CH_3), 1.69 (d, 3a-CH_3, J = 7.1 Hz), 1.63 (d, 8a-CH_3, J = 7.1 Hz), 1.30 (t, 4a-CH_3); ^{252}Cf PDMS, calcd average $[M]^+$ 618.01, [M – $(CH_2)_2OH]^+$ 572.95; observed $[M]^+$ 619.17, $[M - (CH_2)_2OH]^+$ 574.25.

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