Partial Syntheses of Optically Pure Methyl Bacteriopheophorbides *c* and *d* from Methyl Pheophorbide *a*[1]

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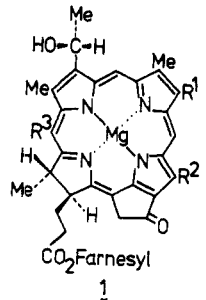
A partial synthesis of the diastereomeric mixture of methyl pheophorbides **2** from the chlorophyll degradation product chlorin e_6 trimethyl ester (**4**) is described. Compound **2** is related to band 6 of the bacteriochlorophylls *c* (*Chlorobium* chlorophylls, "660" series) and also to bacteriochlorophyll c_s recently isolated from *Chloroflexus aurantiacus*. Separation of the *R,S* diastereomeric mixture [at the 2-(1-hydroxyethyl)group] is readily accomplished by using reverse-phase high-performance liquid chromatography (high-performance LC). Markownikoff hydration of methyl pyropheophorbide *a* (**17**) similarly gives the *R,S* diastereomeric mixture [**16(R),16(S)**] which can in turn be separated by high-performance LC. On account of Woodward's earlier total synthesis of optically pure chlorin e_6 trimethyl ester (**4**), this work constitutes a formal total synthesis of the optically pure methyl pheophorbides of band 6 of the bacteriochlorophylls *c* (equivalent to methyl bacteriopheophorbide c_s) and of band 6 of the bacteriochlorophylls *d*.

The bacteriochlorophylls *c*, BChl-c (*Chlorobium* chlorophylls, "660"), **1**, are a homologous group of chlorophylls



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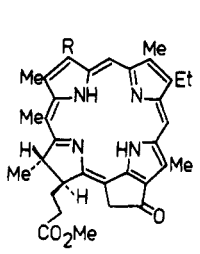
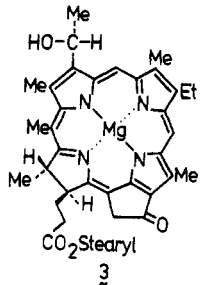
Band	Cmpd.	R^1	R^2	R^3
1	1a	i-Bu	Et	Et
2	1b	i-Bu	Et	Me
3	1c	n-Pr	Et	Et
4	1d	n-Pr	Et	Me
5	1e	Et	Et	Me
6	1f	Et	Me	Me

derived from various green sulfur bacteria.[2] Apart from extra methionine-derived[3] methylations in the 4 and 5 side chains, the main difference between the porphyrin skeleton of the BChl-c and chlorophyll *a* (Chl-a) is the δ-methyl substituent.[4,5] Indeed, for the case of BChl-c (band 6, **1f**), the δ-methyl group is the only major difference since side chains at 4 and 5 are ethyl and methyl, respectively, as they are in Chl-a itself. With our recent report[6] of a procedure for insertion of a methyl group at the δ-position of chlorins (i.e., adjacent to the dihydro subunit), the way was opened for synthesis of band 6 of this series of bacterial chlorophylls from a Chl-a degradation product. The structure **2** was chosen as our synthetic objective; not only is it the



2 R = CH(OH)Me
14 R = CH_2CH_2Cl
15 R = $CH{=}CH_2$



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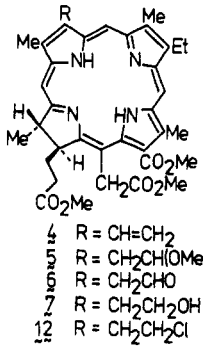
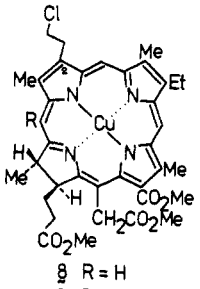


4 R = $CH{=}CH_2$
5 R = $CH_2CH(OMe)_2$
6 R = CH_2CHO
7 R = CH_2CH_2OH
12 R = CH_2CH_2Cl



8 R = H
9 R = CHO
10 R = Me

pheophorbide of BChl-c, band 6, but it is also the pheophorbide corresponding to BChl-c_s (**3**), the main photosynthetic pigment from the phototropic bacterium *Chloroflexus aurantiacus*.[7]

We chose chlorin e_6 trimethyl ester (**4**) as the starting material[8] and, after enduring problems with formylation of the 2-vinyl group,[9] accomplished a synthesis of the mesopheophorbide from band 6.[10] In this paper we describe a successful partial synthesis of the methyl bacteriopheophorbide **2** in which all previous synthetic

(1) Presented at the First International Argentine Meeting on Porphyrins and Porphyrias, October 29–November 1, 1979, Buenos Aires, Argentina; Abstract I.4.

(2) Allen, M. B. In "The Chlorophylls"; Vernon, L. P. Seely, G. R., Eds.; Academic Press: New York, 1966; p 516.

(3) Kenner, G. W.; Rimmer, J.; Smith, K. M.; Unsworth, J. F. *J. Chem. Soc., Perkin Trans. 1* **1978**, 845–52.

(4) Cox, M. T.; Jackson, A. H.; Kenner, G. W. *J. Chem. Soc. C* **1971**, 1974–81.

(5) Chapman, R. A.; Roomi, M. W.; Morton, T. C.; Krajcarski, D. T.; MacDonald, S. F. *Can. J. Chem.* **1971**, *49*, 3544–64.

(6) Bushell, M. J.; Evans, B.; Kenner, G. W.; Smith, K. M. *Heterocycles* **1977**, *7*, 67–72. This procedure required regioselective formylation of copper(II) chlorins using the Vilsmeier procedure. Vilsmeier formylations of porphyrin metal complexes have previously been described: Inhoffen, H. H.; Fuhrhop, J.-H.; Voigt, H.; Brockmann, H., Jr. *Justus Liebigs Ann. Chem.* **1966**, *695*, 133–42. Johnson, A. W.; Oldfield, D. *J. Chem. Soc. C* **1966**, 794–801.

(7) Gloe, A.; Risch, N. *Arch. Microbiol.* **1978**, *118*, 153–6. Risch, N; Brockmann, H., Jr.; Gloe, A. *Justus Liebigs Ann. Chem.* **1979**, 408–18.

(8) Obtained[10] from methyl pheophorbide *a*, which in turn was prepared from the pheophytin *a* and *b* mixture.[11]

(9) Nichol, A. W. *J. Chem. Soc. C* **1970**, 903–10.

(10) Smith, K. M.; Bisset, G. M. F.; Bushell, M. J. *Bioorg. Chem.*, in press.

(11) Kenner, G. W.; McCombie, S. W.; Smith, K. M. *J. Chem. Soc., Perkin Trans. 1* **1973**, 2517–23.

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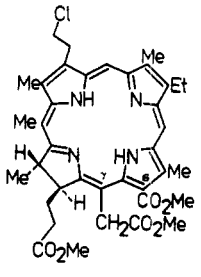
problems have been overcome. Meanwhile, and since publication of our initial paper,[6] Brockmann and coworkers have communicated[12] a synthesis of the same methyl pheophorbide **2**, using a route bearing some similarities to our own.

The vinyl group in chlorin e_6 trimethyl ester (**4**) was protected by using our previously described[13] thallium(III) route, involving treatment with 2 mol of thallium(III) nitrate in methanol (to give **5**) followed by acid treatment (to give **6**) and then borohydride reduction (to give **7** in 85% overall yield). Vilsmeier formylation of the copper(II) complex from **7** with $POCl_3$/DMF accomplished *both* insertion of the δ-formyl group and transformation of the 2-(2-hydroxyethyl) into the 2-(2-chloroethyl) group, affording a 65% yield of **9**. Reduction with tetra-*n*-butylammonium borohydride[10] gave a 72% yield of the mesomethylchlorin **10** along with small quantities of more polar, further reduction products. A more satisfactory reduction of the formyl group to a methyl group was obtained with sodium borohydride in acetic acid,[14] and in this way an 87% yield of **10** was secured. Demetalation with 25% sulfuric acid in trifluoroacetic acid gave the required metal-free compound **11**, along with the δ-unsubstituted



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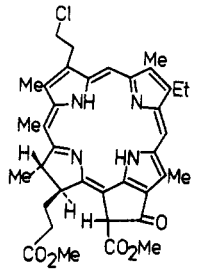

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copper complex **8**. At no time, under a variety of reaction times, was any δ-methyl copper complex **10** or any δ-unsubstituted free base **12** recovered, and from this it was deduced that the large concentrations of sulfuric acid present were cleaving the δ-methyl group from the chlorin and that the copper complex **8** was stable to 25% H_2SO_4 in TFA. On the other hand, because the metal-free δ-substituted chlorin **11** was obtained, we reasoned that the complex **10** was rapidly demetalated under these conditions. Use of 1% H_2SO_4 in TFA gave an 88% yield of the required demetalated product **11** without production of the other compounds previously evident under the more vigorous (25% H_2SO_4) conditions. The ready demetalation of **10** (compared with **8**) is presumably a consequence of the porphyrin core distortion caused by the steric effect of the δ-methyl group in **10**; such buckling of the porphyrin ring is known[15] to aid demetalation.

Cyclization of the 6- and γ-substitutents in **11** to give the pheophorbide **13** was accomplished with *tert*-butoxide under rigorously oxygen-free conditions,[16] and yields near 100% were usually obtained. Heating in collidine[11] gave the pyropheophorbide **14** (97% yield) which was treated

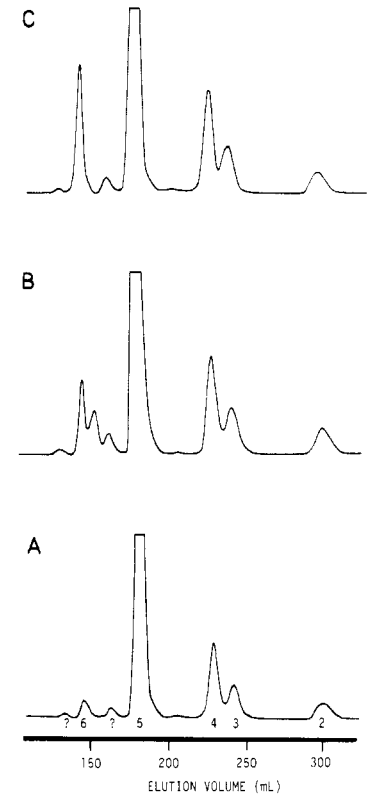


Figure 1. High-performance LC chromatograms: (A) The natural mixture of methyl pheophorbides from *Chloropseudomonas ethylicum*; numbers beneath peaks represent band numbers as indicated in 1. Structures of pigments in bands ? and 3 remain unknown. (B) The natural mixture doped with an aliquot of synthetic methyl pheophorbide mixture **2**. (C) The natural mixture, as in A, but doped with an aliquot of the high-performance LC separated 2(*R*)-(1-hydroxyethyl) diastereomer **2(R)**. High-performance LC conditions: Waters Associates ALC/GPC-201 instrument with 405-nm detector; 2 μBondapak C-18 reverse-phase columns (30 cm × 7.8 mm i.d.); solvent was 15% H_2O in MeOH, with flow rate 3 mL/min and ~2000 psi back pressure.

with potassium hydroxide in pyridine to give the vinyl analogue **15** in 93% yield. Finally, treatment with HBr in acetic acid gave a 49% yield of the required 2-(1-hydroxyethyl)pheophorbide **2**, obtained as a mixture (vide infra) of diastereomers at the 2-position.

The identity of our synthetic material was established by subjecting it to high-performance liquid chromatography. Figure 1A shows our reverse-phase separation[17] of the natural mixture of methyl pheophorbides from *Chloropseudomonas ethylicum*, and Figure 1B displays the same mixture, doped with our synthetic 2(*R,S*)-(1-hydroxyethyl) mixture **2**. Unexpectedly, Figure 1B shows, along with the expected enlargement of the band 6 peak, a new, slightly slower running peak. The 360-MHz NMR spectrum (Figure 2A) also showed two components to be present and it seems logical to assume that these chromatographically and spectroscopically discrete entities are the *R* and *S* forms of the 2-(1-hydroxyethyl)bacteriopheophorbides **2(R)** and **2(S)**, respectively. High-performance LC analysis of the synthetic mixture **2** (Figure 3A) confirmed this interpretation, with the more mobile *R* form[18] predominating in an apparently 3:2 mixture, assuming identical extinction

(12) Brockmann, H., Jr.; Jürgens, U.; Thomas, M. *Tetrahedron Lett.* **1979**, 2133–6.
(13) Kenner, G. W.; McCombie, S. W.; Smith, K. M. *Justus Liebigs Ann. Chem.* **1973**, 1329–38.
(14) Fieser L. F.; Fieser, M. "Reagents for Organic Synthesis"; Wiley: New York, 1975; Vol. 5, p 601.
(15) Hambright, P. In "Porphyrins and Metalloporphyrins"; Smith, K. M., Ed.; Elsevier: Amsterdam, 1975; Chapter 6.
(16) If oxygen was allowed to enter the reaction vessel prior to quenching of the base, large amounts of the corresponding δ-methylpurpurin-18 methyl ester and δ-methyl-2-(2-chloroethyl)-2-devinylpurpurin-18 methyl ester were obtained.
(17) Smith, K. M.; Bushell, M. J.; Rimmer, J.; Unsworth, J. F. *J. Am. Chem. Soc.* **1980**, *102*, 2437–48.
(18) The natural configuration at the 2-(1-hydroxyethyl) group in bacteriochlorophylls *c*, *d*, and *e*, has been shown to be *R*. Risch, N.; Brockmann, H., Jr. *Justus Liebigs Ann. Chem.* **1976**, 578–83.

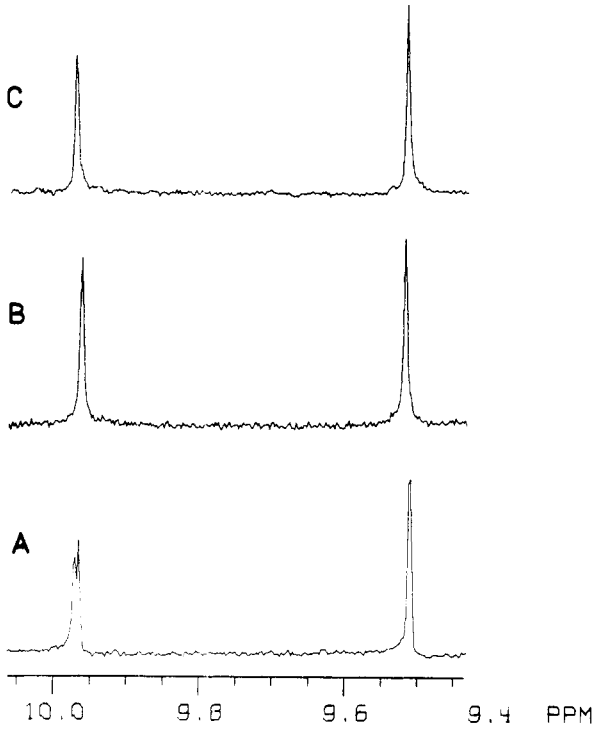


Figure 2. Proton NMR spectra (360 MHz) of the methine-proton region in (A) the synthetic *R,S* mixture of methyl pheophorbides **2**, (B) the high-performance LC separated (most mobile) diastereomer **2(R)**, and (C) the high-performance LC separated (least mobile) diastereomer **2(S)**.

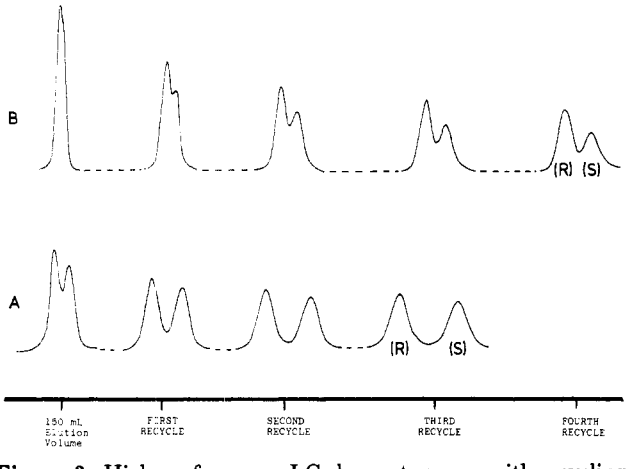
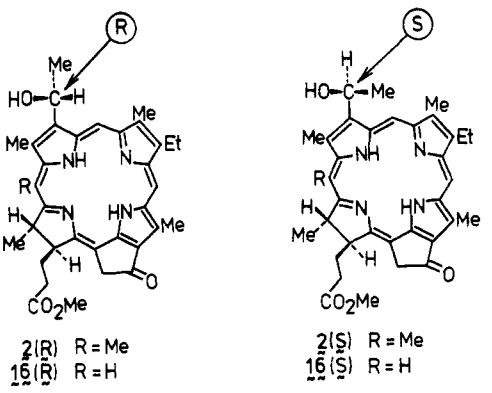


Figure 3. High-performance LC chromatograms, with recycling, of (A) the synthetic *R,S* methyl pheophorbide mixture **2** and (B) the synthetic *R,S* methyl pheophorbide mixture **16(R)** and **16(S)**. Chromatography conditions are as described for Figure 1.

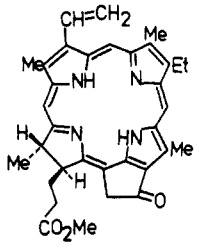
coefficients for the two components. After preparative high-performance LC separation, the natural mixture was once again doped with compound **2(R)**, and the expected enhancement of band 6 (Figure 1C) was achieved. Figures 2B and 2C show the NMR spectra (methine-proton region) of the separated diastereomers.

A similar synthesis of the analogous optically pure methyl bacteriopheophorbide (**16**) was readily accomplished by Markownikoff hydration of methyl pyropheophorbide *a* (**17**). High-performance LC separation (Figure 3B) of the *R* and *S* 2-(1-hydroxyethyl) compounds **16(R)** and **16(S)** was accomplished as previously and the preparative separation was again confirmed by the 360-MHz NMR spectra (Figure 4); unequal formation of the two diastereomers was again suggested by high-performance LC (Figure 3B) (assuming equal extinction coefficients),



2(R) R = Me
16(R) R = H

2(S) R = Me
16(S) R = H



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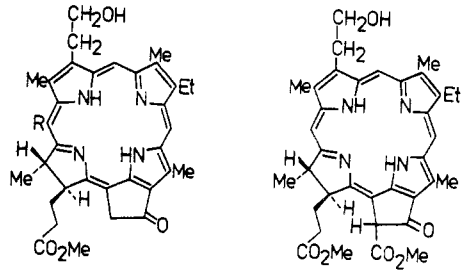
though NMR spectra (Figure 4A) did not indicate this inequality.[19]

One final concern was that the second peak in both chromatograms (Figure 3) might be due to something other than the *S* diastereomers **2(S)** and **16(S)** and that the *R,S* mixture might be co-chromatographing at the retention volume assigned to the pure diastereomers **2(R)** and **16(R)**. One likely candidate[21] for the slow-running material is the 2-(2-hydroxyethyl) compound **18** (or **19**) for the bacterio-



18 R = Me
19 R = H

20

pheophorbide *c* (bacteriopheophorbide *d*) series. To eliminate this possibility, we synthesized **19** from the 2-(2-hydroxyethyl)chlorin e_6 trimethyl ester **7** by anaerobic treatment with potassium *tert*-butoxide (to give the pheophorbide **20**) and then heating in collidine to give[11] the required pyropheophorbide **19**. When the material was mixed with the proposed (*R,S*)-bacteriopheophorbides *d* (**16**), three peaks were clearly observed upon high-performance LC analysis with recycling, the material **20** being less mobile than either **16(R)** or **16(S)**.

(19) By analogy with the **2(R,S)** series the *R* form would be expected to have the highest field α proton (Figure 4). This assumption is confirmed by a partial resolution of the *R,S* mixture accomplished by Risch and Reich[20] in which the *R* diastereomer was enriched to the extent of about 70%.

(20) Risch, N.; Reich, H. *Tetrahedron Lett.* **1979**, 4257–60.

(21) Deuterium exchange reactions have implicated primary carbonium ion formation during protonation of porphyrinic vinyl groups. Smith, K. M.; Langry, K. C. *J. Chem. Soc., Chem. Commun.* **1979**, 1001–3.

On the basis of Woodward's previous total synthesis[22] of optically pure chlorin e_6 trimethyl ester (**4**), the work herein constitutes formal total syntheses of the band 6[2] methyl bacteriopheophorbides *c* and *d*. Recently, Brockmann et al.[12] have communicated a synthesis of the *R,S* mixture **2** (from **4**), but no attempt was made to separate the diastereomers. More recently still, Risch and Reich[20] have published a note showing that the methyl bacteriopheophorbides *d* **16(R)** and **16(S)** can be partially enriched in the *R* form, and eventually separated, by chromatography on silica gel coated with the charge-transfer agent[23] (*S*)-(+)-2-(2,4,5,7-tetranitro-*O*-fluorenylideneaminooxy)-propionic acid. Normal-phase high-performance LC was unsuccessful when applied to this separation.

Experimental Section

Melting points were measured on a microscopic hot-stage apparatus. TLC monitoring of all reactions was performed with Merck silica gel 60 F254 precoated sheets (0.2 mm), and preparative TLC separations were carried out on 20 × 20 cm glass plates coated with Merck GF 254 silica gel (1.5 mm). Column chromatography was performed with Merck neutral alumina 90 (70–230 mesh). Electronic absorption spectra were measured with a Cary-17 spectrophotometer (solutions in methylene chloride), and ^{1}H NMR spectra were determined in deuteriochloroform solution with tetramethylsilane as internal standard at either 200 or 360 MHz with a Nicolet NT-200 or NT-360 spectrometer. Mass spectra (direct insertion probe, 70 eV, 50 μA, source temperature ca. 200 °C) were measured with either an AEI MS-12 or a Finnigan 3200 mass spectrometer.

Chlorin e_6 trimethyl ester (**4**) used in this research was obtained[10,11] by classical degradation of a pheophytin *a* and *b* mixture.

2-(2-Hydroxyethyl)-2-devinylchlorin e_6 Trimethyl Ester (7). Chlorin e_6 trimethyl ester (**4**)[10] (479 mg) was dissolved in 100 mL of methylene chloride and stirred at 40 °C while 730 mg of thallium(III) trinitrate trihydrate was added in 35 mL of dry methanol. The mixture was stirred for 25 min before sulfur dioxide gas was bubbled through the solution during 10 s. Then, 0.5 mL of concentrated hydrochloric acid was added and the solution was poured into water; the organic extracts were separated and washed with more water. The solution was dried (Na_2SO_4) and evaporated to dryness to give a solid which was dissolved in 25 mL of methylene chloride and 100 mL of tetrahydrofuran, to which was added 2 mL of concentrated hydrochloric acid in 3 mL of water. The mixture was heated under reflux for 30 min and then diluted with methylene chloride and water, and the organic layer was washed with water, dried (Na_2SO_4), and evaporated to dryness in vacuo. TLC monitoring showed the esters to be hydrolyzed; the residue was dissolved in 50 mL of methylene chloride and a solution of 1 g of sodium borohydride in 40 mL of methanol was added. The mixture was stirred at room temperature for 1 h before 2 mL of glacial acetic acid was added and the solution was poured into water. After the aqueous phase was extracted with chloroform, the organic layers were combined, washed with saturated aqueous sodium chloride, dried (Na_2SO_4), and evaporated to dryness. The product was then treated with excess ethereal diazomethane, and after it was allowed to stand for a few minutes the solution was evaporated to dryness. Purification by column chromatography (Brockmann grade V alumina, elution with methylene chloride) and subsequent crystallization from methylene chloride/hexane gave the required (2-hydroxyethyl)chlorin (418 mg, 84%): mp 104–105 °C; NMR δ 1.64 (t, 3 H, 4-CH_2CH_3), 1.70 (d, 3 H, 8-CH_3), 2.0–2.9 (m, 4 H, $CH_2CH_2CO_2CH_3$), 3.20, 3.26, 3.54, 3.60, 3.75, 4.23 (each s, 3 H, 1,3,5-CH_3, 7,γ,6-CO_2CH_3), 3.8–4.5 (m, 6 H, 4-CH_2CH_3, 2-CH_2CH_2OH), 4.50 (t, 1 H, 7-H), 4.6 (q, 1 H, 8-H), 5.27 (s, 2 H, γ-$CH_2CO_2CH_3$), 8.68, 9.31, 9.68 (each s, 1 H, δ,α,β-H); mass spectrum, *m/e* 656 (100%, M^+); visible λ_{max} 396 nm (ε 174 500), 496 (14 400), 522 (3850), 550 (2300), 598 (5700), 652 (51 100). Anal. Calcd for $C_{37}H_{44}N_4O_7$: C, 67.66; H, 6.75; N, 8.53. Found: C, 67.81; H, 6.97; N, 8.38. The yield in this reaction was initially found to be variable, but optimum yields can be obtained if all solvents are freshly dried, if the reaction mixture is protected from light, and if care is taken to remove all chelated thallium. Best yields were obtained if, as described above, 2.2 molar equiv of thallium(III) trinitrate trihydrate is used.

Copper(II) δ-Formyl-2-(2-chloroethyl)-2-devinylchlorin e_6 Trimethyl Ester (9). A saturated solution of copper(II) acetate in 2 mL of methanol was added dropwise to a gently refluxing solution of 100 mg of **7** in 30 mL of dichloromethane. Heating was continued until spectrophotometry indicated complete disappearance of the peak at 654 nm (5 min). To this was added 50 mL of methanol and the solution was reduced in volume to 5 mL by evaporation in vacuo. The turquoise precipitate was collected by filtration, purified by column chromatography (Brockmann grade V alumina, elution with methylene chloride), and then crystallized from methylene chloride/methanol to give the copper complex (109 mg, 100%), mass spectrum, *m/e* (^{63}Cu) 717 (100%), 570 (49). A solution of 70 mg of this compound in 50 mL of dry 1,2-dichloroethane was added at 50 °C to a solution of the Vilsmeier complex from 1 mL of phosphoryl chloride and 0.84 mL of dimethylformamide in 20 mL of 1,2-dichloroethane. The mixture was stirred at 50 °C for 1 h and then 200 mL of aqueous saturated sodium acetate was added cautiously, the reaction mixture then being heated at 60 °C for a further 1 h.[24] Extraction with 3 × 50 mL of methylene chloride, washing with 100 mL of saturated aqueous sodium bicarbonate, drying (Na_2SO_4), and evaporation in vacuo afforded a green solid. Purification by column chromatography (Brockmann grade V alumina, elution with chloroform) and recrystallization from methylene chloride/hexane yielded the *meso*-formylchlorin–copper(II) complex **9** (48 mg, 65%): mp 159 °C; mass spectrum *m/e* (^{63}Cu) 763 (100%, M^+), 729 (24), 616 (14); visible λ_{max} 391 nm (ε 70 000), 416 (125 300), 507 (4800), 546 (5200), 623 (12 500), and 663 (43 400). Anal. Calcd for $C_{38}H_{41}ClCuN_4O_7$: C, 59.68; H, 5.40; N, 7.33. Found: C, 59.89; H, 5.65; N, 7.22.

Copper(II) δ-Methyl-2-(2-chloroethyl)-2-devinylchlorin e_6 Trimethyl Ester (10). Method A. A solution of 100 mg of copper complex **9** in 40 mL of 1,2-dichloroethane and 2 mL of ethyl acetate was heated to reflux before treatment with 200 mg of freshly prepared tetra-*N*-butylammonium borohydride. After being refluxed for 10 min, the turquoise solution was quenched with 50 mL of water, extracted with 3 × 50 mL of methylene chloride, washed with 20 mL of 0.1 M hydrochloric acid, dried (Na_2SO_4), and then evaporated in vacuo. Purification by column chromatography (Brockmann grade III alumina, elution with methylene chloride) and crystallization from methylene chlo-

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(23) Mikes, F.; Boshart, G.; Gil-Av, E. *J. Chromatog.* **1976**, *122*, 205–21.

(24) In one experiment, the imine salt hydrolysis, using saturated aqueous sodium acetate, was carried out at 80 °C for more than 2 h. TLC analysis showed considerable quantities of a compound more polar than **9** to be present. The mixture was reduced (as described for **10**, method B) and then demetalated (as described for **11**), and the more polar byproduct, after TLC separation, was shown by mass and NMR spectroscopy to be the corresponding 2-(2-acetoxyethyl) derivative of **11**, presumably produced by nucleophilic displacement of the side-chain chloride by acetate.[27] NMR spectrum of the 2-(2-acetoxyethyl) compound: δ −1.60 (br s, 2 H, NH), 1.56 (d, 3 H, 8-CH_3), 1.72 (m, 4 H, 4-CH_2CH_3 and one H of 7-$CH_2CH_2CO_2CH_3$), 1.72 (t, 3 H, 4-CH_2CH_3), 1.85–2.00 (m, 1 H, $CH_2CH_2CO_2CH_3$), 2.08 (s, 3 H, $CH_2CH_2OCOCH_3$), 2.18–2.33, 2.48–2.64 (each m, 1 H, $CH_2CH_2CO_2CH_3$), 3.33, 3.48, 3.54, 3.62 (each s, 3 H, 3,1,5-CH_3, 7-$CH_2CH_2CO_2CH_3$), 3.76 (q, 2 H, 4-CH_2CH_3), 3.84, 3.86, 4.25 (each s, 3 H, δ-CH_3, γ-$CH_2CO_2CH_3$, 6-CO_2CH_3), 4.15-4.37 (m, 3 H, CH_2CH_2O-$COCH_3$, 7-H), 4.57 (q, 1 H, 8-H), 4.66–4.82 (m, 2 H, $CH_2CH_2OCOCH_3$), 5.01, 5.29 (AB q, 2 H, J_{AB} = 19 Hz, γ-$CHCO_2CH_3$), 9.50, 9.56 (each s, 1 H, α,β-H). This compound was treated with 5% sulfuric acid in methanol for 16 h in the dark to afford the 2-(2-hydroxyethyl) derivative of **11**: NMR δ −1.59 (br s, 2 H, NH), 1.56 (d, 3 H, 8-CH_3), 1.70 (m, 4 H, 4-CH_2CH_3 and one H of 7-$CH_2CH_2CO_2CH_3$), 1.85–2.00 (m, 1 H, 7-$CH_2CH_2CO_2Me$), 2.18–2.32, 2.48–2.64 (each m, 1 H, 7-$CH_2CH_2CO_2CH_3$), 3.29, 3.41, 3.50, 3.62 (each s, 3 H, 3,1,5-CH_3, 7-$CH_2CH_2CO_2CH_3$), 3.75 (q, 2 H, 4-CH_2CH_3), 3.83, 3.85, 4.26 (each s, 3 H, δ-CH_3, γ-$CH_2CO_2CH_3$, 6-CO_2CH_3), 4.12 (t, 2 H, CH_2CH_2OH), 4.21–4.36 (m, 3 H, 7-H, CH_2CH_2OH), 4.57 (q, 1 H, 8-H), 5.00, 5.29 (AB q, 2 H, J_{AB} = 19 Hz, γ-$CH_2CO_2CH_3$), 9.41, 9.55 (each s, 1 H, α,β-H). The 2-(2-hydroxyethyl) derivative was converted into the required 2-(2-chloroethyl)chlorin **11** by treatment with thionyl chloride in dimethylformamide containing a suspension of potassium carbonate.[28]

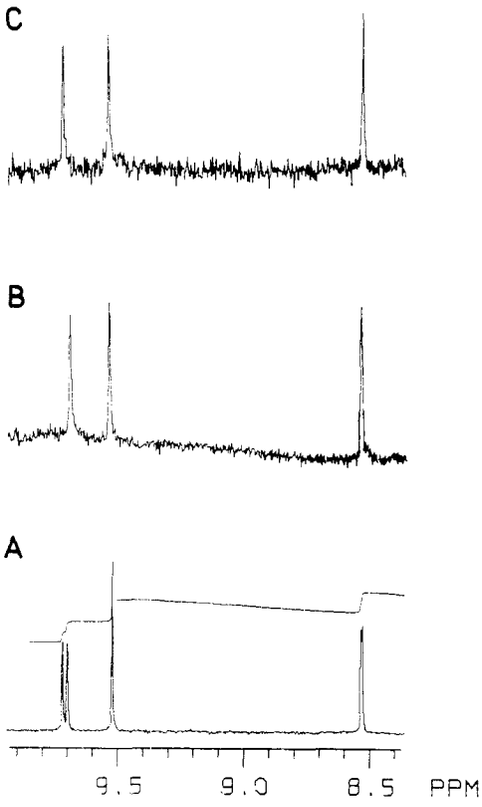

Figure 4. Proton NMR spectra (360 MHz) of the methine proton region in (A) the synthetic *R,S* mixture **16(R,S)** of methyl pheophorbide from hydration of methyl pyropheophorbide (**17**), (B) the high-performance LC separated (most mobile) diastereomer **16(R)**, and (C) the high-performance LC separated (least mobile) diastereomer **16(S)**.

ride/hexane afforded **10** (71 mg; 72%). TLC monitoring showed significant amounts of two more polar compounds to be present at the end of the reduction; these compounds are presumably caused by reduction of the methyl esters during the course of the reaction, so a second more efficient method was developed as follows.

Method B. Glacial acetic acid (20 mL) was cooled to 10 °C and 200 mg of sodium borohydride was cautiously added dropwise. A green solution of 100 mg of **9** in 5 mL of glacial acetic acid was added to the borohydride solution while maintaining the temperature at 10 °C. After 10 min the resulting turquoise solution was cautiously quenched with 100 mL of water and extracted with 3 × 100 mL of methylene chloride, and the extracts were washed with 30 mL of water and then 50 mL of 1% aqueous sodium carbonate. The methylene chloride phase was dried (Na_2SO_4) and evaporated to dryness to give a residue which was purified as in method A. The yield of **10** was 85 mg (87%): mp 98 °C; mass spectrum, *m/e* (^{63}Cu) 749 (100%, M^+), 714 (9), 689 (11), 601 (28), 589 (31), 539 (32); visible λ_{max} 414 nm (ϵ 133 000), 508 (4300), 554 (4500), 598 (9700), and 639 (42 700). Anal. Calcd for $C_{38}H_{43}ClCuN_4O_6$: C, 60.79; H, 5.77; N, 7.46. Found: C, 60.58; H, 5.80; N, 7.25.

δ-Methyl-2-(2-chloroethyl)-2-devinylchlorin e_6 Trimethyl Ester (11). The foregoing copper(II) chlorin **10** (250 mg) was dissolved in a mixture of 50 mL of trifluoroacetic acid and 0.5 mL of concentrated sulfuric acid and then stirred at 25 °C for 1 h in the dark. The mixture was then poured onto 200 mL of iced water and the aqueous solution was extracted with 3 × 100 mL of methylene chloride. After the organic phase had been washed with saturated aqueous sodium carbonate and water, it was dried (Na_2SO_4) and evaporated to dryness. Purification was achieved by column chromatography (Brockmann grade III alumina, elution with methylene chloride) and subsequent recrystallization from methylene chloride/methanol gave **11** as light brown crystals (203 mg, 88%): mp 130–131 °C; NMR δ −1.61 (br s, 2 H, NH), 1.56 (d, 3 H, 8-CH_3), 1.70 (t, 3 H, 4-CH_2CH_3), 3.15–2.42, 2.42–2.66 (each m, 2 H, 7-$CH_2CH_2CO_2CH_3$), 3.30, 3.42, 3.54, 3.62 (each s, 3 H, 3,1,5-CH_3, 7-$CH_2CH_2CO_2CH_3$), 3.75 (q, 2 H, 4-CH_2CH_3), 3.82, 3.85, 4.25 (each s, 3 H, δ-CH_3, γ,6-CO_2CH_3), 4.07–4.23, 4.24–4.43 (each m, 2 H and 3 H, CH_2CH_2Cl, 7-H), 4.57 (q, 1 H, 8-H), 5.00, 5.31 (AB q, J_{AB} = 19 Hz, 2 H, γ-$CH_2CO_2CH_3$), 9.38, 9.56 (each s, 1 H, α,β-H); mass spectrum, *m/e* 688 (100%, M^+), 656 (20), 629 (40), 616 (45), 601 (30), 529 (75); visible λ_{max} 404 nm (ϵ 187 000), 508 (12 600), 536 (8800), 612 (5000), and 666 (49 000). Anal. Calcd for $C_{38}H_{45}ClN_4O_6$: C, 66.22; H, 6.58; N, 8.13. Found: C, 66.31; H, 6.62; N, 8.24.

δ-Methyl-2-(2-chloroethyl)-2-devinylpheophorbide *a* Methyl Ester (13). The foregoing trimethyl ester **11** (174 mg) was dissolved in 10 mL of dry pyridine and then carefully degassed with nitrogen at 50 °C. A degassed solution of potassium *tert*-butoxide in *tert*-butyl alcohol (3 mL of a mixture of 200 mg of potassium metal in 10 mL of *tert*-butyl alcohol) was added. The initial bright green color turned orange after 2 min of stirring at 50 °C under nitrogen (failure to observe the orange species usually was a prelude to a low yield). After 15 min, the orange solution was quenched with 3 mL of degassed glacial acetic acid, poured into water, and extracted with 3 × 100 mL of methylene chloride. The organic phase was washed with 50 mL of 2 M HCl and 100 mL of water and dried (Na_2SO_4). Evaporation to dryness gave a residue which was treated with excess ethereal diazomethane, evaporated, and purified by column chromatography (Brockmann grade V alumina, elution with 1:1 methylene chloride–hexane). Recrystallization from methylene chloride/methanol gave brown crystals of the pheophorbide **13** (160 mg, 97%): mp 198–200 °C; NMR δ −1.71 (br s, 2 H, NH), 1.51 (d, 3 H, 8-CH_3), 1.70 (t, 3 H, 4-CH_2CH_3), 2.10–2.29, 2.30–2.55 (each m, 2 H, 7-$CH_2CH_2CO_2CH_3$), 3.29, 3.47, 3.52, 3.69 (each s, 3 H, 3,1,5-CH_3, 7-$CH_2CH_2CO_2CH_3$), 3.73 (q, 2 H, 4-CH_2CH_3), 3.85, 3.88 (each s, 3 H, δ-CH_3, γ-$CHCO_2CH_3$), 4.13–4.25, 4.33–4.42 (each m, 3 H, 2 H, 7-H, CH_2CH_2Cl), 4.56 (q, 1 H, 8-H), 6.23 (s, 1 H, γ-$CHCO_2CH_3$), 9.33, 9.52 (each s, 1 H, α,β-H); mass spectrum, *m/e* 656 (100%, M^+), 624 (20), 597 (55), 569 (19), 509 (47), 494 (27), 459 (23), 446 (30); visible λ_{max} 415 nm (ϵ 141 500), 516 (12 500), 549 (17 500), 613 (9100), and 670 (62 300). Anal. Calcd for $C_{37}H_{41}ClN_4O_5$: C, 67.62; H, 6.29; N, 8.53. Found: C, 68.32; H, 6.22; N, 8.69. If the orange color was not observed or was not maintained for long after addition of the *tert*-butoxide, the major products (λ_{max} 708 nm) (characterized by their 360-MHz NMR spectra) were[16] the following: **δ-methylpurpurin-18 methyl ester** [NMR 1.64 (t, 3 H, 4-CH_2CH_3), 1.67 (d, 3 H, 8-CH_3), 1.88–2.80 (m, 4 H, $CH_2CH_2CO_2CH_3$), 3.14, 3.36, 3.56 (each s, 3 H, 3,1,5-CH_3), 3.60 (q, 2 H, 4-CH_2CH_3), 3.72, 3.75 (each s, 3 H, δ-CH_3, 7-$CH_2CH_2CO_2CH_3$), 4.49 (d of q, 1 H, 8-H), 5.14 (d of t, 1 H, 7-H), 6.09, 6.24 (m, 2 H, vinyl CH_2), 7.77, 7.83 (m, 1 H, vinyl CH), 9.39, 9.50 (each s, α,β-H); mass spectrum, *m/e* 592 (66%), 505 (100), 461 (26)] and **δ-methyl-2-(2-chloroethyl)-2-devinylpurpurin-18 methyl ester** [NMR 1.38 (t, 3 H, 4-CH_2CH_3), 1.67 (d, 3 H, 8-CH_3), 1.88–2.80 (m, 4 H, 7-$CH_2CH_2CO_2CH_3$), 3.18, 3.34, 3.56 (each s, 3 H, 3,1,5-CH_3), 3.59 (q, 2 H, CH_2CH_3), 3.74, 3.76 (each s, 3 H, δ-CH_3, 7-$CH_2CH_2CO_2CH_3$), 4.05–4.30 (m, 4 H, CH_2CH_2Cl), 4.48 (d of t, 1 H, 8-H), 5.14 (d of t, 1 H, 7-H), 9.29, 9.54 (each s, 1 H, α,β-H); mass spectrum, *m/e* (^{35}Cl) 628 (17%), 541 (26), and 505 (100)].

δ-Methyl-2-(2-chloroethyl)-2-devinylpyropheophorbide *a* Methyl Ester (14). The pheophorbide **13** (18 mg) in 10 mL of dry collidine was heated under reflux at 190 °C for 2 h under an atmosphere of nitrogen in the dark. The collidine was evaporated under high vacuum and the residue was purified by preparative TLC (silica gel, elution with 5% methanol in methylene chloride). After extraction from the silica gel with 10% methanol in methylene chloride, the product was crystallized from methylene chloride/methanol, giving **14** (16 mg, 97%): mp 212–213 °C; NMR δ −1.73 (br s, 2 H, NH), 1.53 (d, 3 H, 8-CH_3), 1.72 (t, 3 H, 4-CH_2CH_3), 2.12–2.28, 2.43–2.62 (each m, 2 H, 7-$CH_2CH_2CO_2CH_3$), 3.32, 3.49, 3.58, 3.71 (each s, 3 H, 3,1,5-CH_3, 7-$CH_2CH_2CO_2CH_3$), 3.74 (q, 2 H, 4-CH_2CH_3), 3.93 (s, 3 H, δ-CH_3), 4.15–4.29 (m, 3 H, 7-H, CH_2CH_2Cl), 4.40 (t, 2 H, CH_2CH_2Cl), 4.61 (q, 1 H, 8-H), 5.21, 5.29 (AB q, J_{AB} = 23 Hz, 2 H, γ-CH_2CO), 9.35, 9.53 (each s, 1 H, α,β-H); mass spectrum, *m/e* 589 (100%, M^+), 562 (38), 511 (29), 475 (16), 447 (20); visible λ_{max} 414 nm (ϵ 128 500), 484 (4400), 518 (11 200), 549 (15 700), 612 (7700), and 669 (54 900). Anal. Calcd for $C_{35}H_{39}ClN_4O_3$: C, 70.16; H, 6.56; N, 9.35. Found: C, 70.40; H, 6.19; N, 9.49.

δ-Methylpyropheophorbide *a* Methyl Ester (15). The foregoing pyropheophorbide **14** (16 mg) was dissolved in 10 mL of pyridine and degassed under nitrogen. To this boiling solution was added 2 mL of water, and, after a further 5 min, 2 mL of a solution of 0.6 g of sodium hydroxide in 20 mL of water was also added, keeping the mixture the whole time under nitrogen. The solution was heated under reflux for 1.5 h in the dark and was then quenched by addition of 2 mL of glacial acetic acid. The mixture was diluted with 100 mL of water and extracted into methylene chloride (3 × 100 mL), and the combined organic extracts were washed with 50 mL of water and then dried (Na_2SO_4). The residue obtained by evaporation was treated with excess ethereal diazomethane and evaporated, and the residue was chromatographed on thick-layer plates (silica gel, elution with 5% methanol in methylene chloride). After extraction from the silica gel with 10% methanol in methylene chloride, the product was crystallized from methylene chloride/methanol, yielding **15** as brown crystals (14 mg, 93%): mp 194–195 °C; NMR δ −1.68 (br s, 2 H, NH), 1.52 (d, 3 H, 8-CH_3), 1.70 (t, 3 H, 4-CH_2CH_3), 2.10–2.27, 2.46–2.58 (each m, 2 H, 7-$CH_2CH_2CO_2CH_3$), 3.26, 3.50, 3.57, 3.68 (each s, 3 H, 3,1,5-CH_3, 7-$CH_2CH_2CO_2CH_3$), 3.71 (q, 2 H, 4-CH_2CH_3), 3.91 (s, 3 H, δ-CH_3), 4.21 (t, 1 H, 7-H), 4.59 (q, 1 H, 8-H), 5.22, 5.28 (AB q, J_{AB} = 20 Hz, γ-CH_2CO), 6.13, 6.26 (ABX, 2 H, J_{AB} = 1.4, J_{AX} = 17.7, J_{BX} = 11.5 Hz, 2-vinyl CH_2), 7.96 (ABX, 1 H, 2-vinyl CH), 9.489, 9.491 (each s, 1 H, α,β-H);[25] mass spectrum, *m/e* 562 (100%, M^+), 475 (34), 460 (18), 445 (10), 430 (11); visible λ_{max} 416 nm (ε 115 100), 486 (4100), 520 (10 500), 552 (14 300), 614 (7200), and 672 (49 400). Anal. Calcd for $C_{35}H_{38}N_4O_3$: C, 74.70; H, 6.81; N, 9.96. Found: C, 74.45; H, 7.02; N, 9.72.

δ-Methyl-2-(1-hydroxyethyl)-2-devinylpyropheophorbide *a* Methyl Ester (2). The foregoing vinylpheophorbide **15** (18 mg) was dissolved in 5 mL of a solution of 40% hydrogen bromide in acetic acid and then stirred for 3 h at 55 °C under nitrogen in the dark. The solution was then poured into 100 mL of water and extracted with 3 x 100 mL of methylene chloride. The combined extracts were washed with 100 mL of 10% aqueous sodium carbonate and then 100 mL of water and dried (Na_2SO_4). After evaporation, the residue was treated with excess ethereal diazomethane and evaporated, and the resulting solid was purified by thick-layer chromatography (silica gel, elution with 5% methanol in methylene chloride). The product was extracted from the silica gel with 10% methanol in methylene chloride; it was not possible to crystallize the product, probably because it is a mixture of diastereomers (see text). Yield was 9 mg (49%): NMR δ −1.83/−1.81 (br s, 2 H, NH), 1.52/1.54 (d, 3 H, 8-CH_3), 1.70 (t, 3 H, 4-CH_2CH_3), 2.18 (d, 3 H, 2-CH(OH)CH_3), 2.35 (t, 2 H, $CH_2CH_2CO_2CH_3$), 2.45–2.59 (m, 2 H, $CH_2CH_2CO_2CH_3$), 3.29 (s, 3 H, 3-CH_3), 3.52/3.53 (s, 3 H, 1-CH_3), 3.56/3.57 (s, 3 H, 5-CH_3), 3.68 (s, 3 H, $CH_2CH_2CO_2CH_3$), 3.71 (q, 2 H, 4-CH_2CH_3), 3.89 (s, 3 H, δ-CH_3), 4.01 (t, 1 H, 7-H), 4.59 (q, 1 H, 8-H), 5.24/5.25 (q, 2 H, γ-CH_2CO), 6.56/6.57 (q, 1 H, 2-CH(OH)CH_3), 9.50 (s, 1 H, β-H), 9.955/9.960 (s, 1 H, α-H).[25] After high-performance LC separation (three 2-mg runs) using two μBondapak C-18 columns, multiple recycle, 15% water in methanol (see Figure 3A), the 2-(1-hydroxyethyl) diastereomer **2(R)** (2 mg) was separated and had the following spectroscopic properties: NMR δ −1.83 (br s, 2 H, NH), 1.54 (d, 3 H, 8-CH_3), 1.72 (t, 3 H, 4-CH_2CH_3), 2.17 (d, 3 H, 2-CH(OH)CH_3), 2.35 (t, 2 H, $CH_2CH_2CO_2CH_3$), 2.45–2.59 (m, 2 H, $CH_2CH_2CO_2CH_3$), 3.29 (s, 3 H, 3-CH_3), 3.53 (s, 3 H, 1-CH_3), 3.56 (s, 3 H, 5-CH_3), 3.68 (s, 3 H, $CH_2CH_2CO_2CH_3$), 3.71 (q, 2 H, 4-CH_2CH_3), 3.89 (s, 3 H, δ-CH_3), 4.01 (t, 1 H, 7-H), 4.59 (q, 1 H, 8-H), 5.25 (q, 2 H, γ-CH_2CO), 6.57 (q, 1 H, 2-CH(OH)CH_3), 9.50 (s, 1 H, β-H), 9.96 (s, 1 H, α-H);[25] visible λ_{max} (relative intensity) 416 nm (100), 518 (9), 548 (13), 611 (6), 668 (48); mass spectrum, *m/e* mass spectrum, 580 (40%, M^+), 562 (100), 547 (10), 493 (10), 475 (20). These data, along with reverse-phase high-performance LC analysis (Figure 1C), identified this material with the natural band 6 methyl bacteriopheophorbide *c* from *Chloropseudomonas ethylicum* and, by analogy, with the methyl bacteriopheophorbide c_s from *Chloroflexus aurantiacus*.[7] With the exception of high-performance LC retention time, the physical data for the *S* diastereomer **2(S)** were very similar.

2-(1-Hydroxyethyl)-2-devinylpyropheophorbide *a* Methyl Ester [16(R),16(S)]. This compound was prepared from methyl pyropheophorbide *a*[11] (**17**), in 50% yield, in the same manner as for **2**: mp 245–247 °C (lit.[26] mp 258–261 °C); NMR δ −1.85 (br s, 2 H, NH), 1.69 (t, 3 H, 4-CH_2CH_3), 1.76/78 (d, 3 H, 8-CH_3), 2.14 (d, 3 H, CH(OH)CH_3), 2.17–2.40, 2.40–2.80 (each m, 2 H, 7-$CH_2CH_2CO_2CH_3$), 3.24 (s, 3 H, 3-CH_3), 3.39/40 (s, 3 H, 1-CH_3), 3.60/61 (s, 3 H, 5-CH_3), 3.64 (s, 3 H, 7-$CH_2CH_2CO_2CH_3$), 3.70 (q, 2 H, 4-CH_2CH_3), 4.25 (t, 1 H, 7-H), 4.45 (q, 1 H, 8-H), 5.06/08, 5.24/26 (AB q, 2 H, J_{AB} = 19 Hz, γ-CH_2CO), 6.41/43 (q, 1 H, CH(OH)CH_3), 8.50/51 (s, 1 H, δ-H), 9.48 (s, 1 H, β-H), 9.65/69 (s, 1 H, α-H); mass spectrum, *m/e*, 566 (100%, M^+), 548 (9), 479 (23), 451 (8), 435 (16); visible λ_{max} 396 nm (ε 91 700), 409 (114 600), 504 (10 500), 535 (10 500), 604 (8300), 656 (42 000). Anal. Calcd for $C_{34}H_{38}N_4O_4 \cdot {}^1/_2H_2O$: C, 70.93; H, 6.83; N, 9.73. Found: C, 71.12; H, 6.99; N, 9.76. This mixture was separated by reverse-phase high-performance LC using conditions identical with those for the separation of the mixture **2**. Figure 3B shows the separation achieved upon recycling. In three 2-mg runs, the material was separated and the more mobile fraction was shown to be the *R* form at the 2-(1-hydroxyethyl) group by analogy with NMR data from the **2(R,S)** series and with data of Risch and Reich.[20] **16(R)**: NMR −1.85 (br s, 2 H, NH), 1.69 (t, 3 H, 4-CH_2CH_3), 1.77 (t, 3 H, 8-CH_3), 2.14 (d, 3 H, 2-CH(OH)CH_3), 2.17–2.40, 2.40–2.80 (each m, 2 H, $CH_2CH_2CO_2CH_3$), 3.24 (s, 3 H, 3-CH_3), 3.40 (s, 3 H, 1-CH_3), 3.60 (s, 3 H, 5-CH_3), 3.64 (s, 3 H, $CH_2CH_2CO_2CH_3$), 3.70 (q, 2 H, 4-CH_2CH_3), 4.25 (t, 1 H, 7-H), 4.45 (q, 1 H, 8-H), 5.06, 5.24 (AB q, 2 H, J_{AB} = 19 Hz, γ-CH_2CO), 6.41 (q, 1 H, 2-CH(OH)CH_3), 8.50 (s, 1 H, δ-H), 9.50 (s, 1 H, β-H), 9.66 (s, 1 H, α-H); mass spectrum, *m/e* 566 (100%), 548 (27), 479 (43), 451 (10), 435 (20). **16(S)**: NMR δ −1.79 (br s, 2 H, NH), 1.69 (t, 3 H, 4-CH_2CH_3), 1.78 (t, 3 H, 8-CH_3), 2.14 (d, 3 H, 2-CH(OH)CH_3), 2.17–2.40, 2.40–2.80 (each m, 2 H, $CH_2CH_2CO_2CH_3$), 3.25 (s, 3 H, 3-CH_3), 3.39 (s, 3 H, 1-CH_3), 3.59 (s, 3 H, 5-CH_3), 3.64 (s, 3 H, $CH_2CH_2CO_2CH_3$), 3.70 (q, 2 H, 4-CH_2CH_3), 4.25 (t, 1 H, 7-H), 4.45 (q, 1 H, 8-H), 5.08, 5.24 (AB q, 2 H, J_{AB} = 19 Hz, γ-CH_2CO), 6.43 (q, 1 H, 2-CH(OH)CH_3), 8.51 (s, 1 H, δ-H), 9.50 (s, 1 H, β-H), 9.69 (s, 1 H, α-H); mass spectrum, *m/e* 566 (100%), 548 (20), 479 (30), 451 (12), 435 (20).

2-(2-Hydroxyethyl)-2-devinylpyropheophorbide *a* Methyl Ester (19). The 2-(2-hydroxyethyl)chlorin e_6 trimethyl ester **7** was transformed in 64% yield into the pheophorbide **20** by following the procedure outlined above for the formation of **13** from **11**. **20**: NMR δ −1.64 (s, 2 H, NH), 1.67 (t, 3 H, 4-CH_2CH_3), 1.78 (d, 3 H, 8-CH_3), 2.10–2.35, 2.40–2.65 (each m, 2 H, 7-$CH_2CH_2CO_2CH_3$), 3.23, 3.33, 3.55, 3.65 (each s, 3 H, 3,1,5-CH_3, 7-$CH_2CH_2CO_2CH_3$), 3.67 (q, 2 H, 4-CH_2CH_3), 3.86 (s, 3 H, γ-$CHCO_2CH_3$), 4.08, 4.34 (each t, 2 H, CH_2CH_2OH), 4.20 (t, 1 H, 7-H), 4.42 (q, 1 H, 8-H), 6.20 (s, 1 H, γ-$CHCO_2CH_3$), 8.49, 9.23, 9.49 (each s, 1 H, δ,α,β-H); mass spectrum *m/e* 624 (5%, M^+), 566 (100), 548 (42), 535 (32), 479 (45). **20** was then converted into the corresponding pyropheophorbide **19** in 90% yield by refluxing in collidine as described above in the preparation of **14** from **13**. The product (**19**) was purified by preparative TLC (silica gel, elution with 5% methanol in methylene chloride), and after extraction from the silica gel using 10% methanol in methylene chloride, the product was crystallized from methylene chloride/methanol to give crystals: mp 196–197 °C; NMR δ −1.72 (s, 2 H, NH), 1.67 (t, 3 H, 4-CH_2CH_3), 1.78 (d, 3 H, 8-CH_3), 2.15–2.30, 2.45–2.60 (each m, 2 H, 7-$CH_2CH_2CO_2CH_3$), 3.24, 3.34 (each s, 3 H, 3,1-CH_3), 3.60 (s, 6 H, 5-CH_3, 7-$CH_2CH_2CO_2CH_3$), 3.67 (q, 2 H, 4-CH_2CH_3), 4.09, 4.35 (each t, 2 H, CH_2CH_2OH), 4.22 (t, 1 H, 7-H), 4.43 (q, 1 H, 8-H), 5.02, 5.17 (AB q, 2 H, J_{AB} = 21 Hz, γ-CH_2CO), 8.48, 9.24, 9.43 (each s, 1 H, δ,α,β-H); mass spectrum, *m/e* 566 (100%, M^+), 535 (13), 479 (43), 451 (13), 433 (16); visible λ_{max} 395 nm (ε 93 200), 408 (119 300), 472 (4100), 504

(25) Differentiation between the α and β protons was achieved by using the fact that the α proton can be regioselectively exchanged in deuterioacetic acid: See ref 3 and Smith, K. M.; Unsworth, J. F. *Tetrahedron* **1975**, *31*, 367–75.

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(10 800), 533 (10 400), 601 (8950), 659 (51 800). Anal. Calcd for $C_{34}H_{38}N_4O_4$: C, 72.06; H, 6.76; N, 9.89. Found: C, 72.20; H, 6.73; N, 10.13.

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Registry No. (*R*)-**2**, 59924-02-6; (*S*)-**2**, 73365-61-4; **4**, 55721-87-4; **7**, 73347-54-3; **7**, copper complex, 72415-74-8; **9**, 72415-75-9; **10**, 72415-77-1; **11**, 73347-55-4; **11**, 2-(2-acetoxyethyl) derivative, 73347-56-5; **11**, 2-(2-hydroxyethyl) derivative, 73347-57-6; **13**, 73333-65-0; **14**, 73333-66-1; **15**, 68528-79-0; (*R*)-**16**, 59954-18-6; (*S*)-**16**, 61665-26-7; **17**, 6453-67-4; **19**, 66230-00-0; **20**, 73347-58-7; δ-methylpurpurin-18 methyl ester, 73333-67-2; δ-methyl-2-(2-chloroethyl)-2-devinylpurpurin-18 methyl ester, 73347-59-8.

Total Synthesis of (-)-Aplysin and (-)-Debromoaplysin

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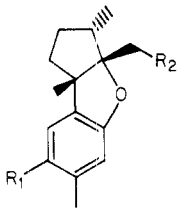
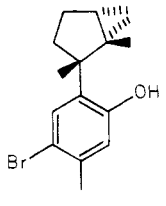
The total synthesis of optically active (-)-aplysin (**1**) and (-)-debromoaplysin (**2**) employing novel (isopinocampheyloxy)methyl ethers for phenolic hydroxyl protection and diastereomeric resolution is described. A key transformation is the unusual cyclization of the diastereomeric chlorohydrins **12** and **13** in methanolic base to form the enantiomeric tricyclic alcohols (-)-**16** and (+)-**16** with cleavage of the acetal-linked (isopinocampheyloxy)methyl resolving/protecting group. This transformation was followed by substitution of the tertiary hydroxyl via the derived chloride with a methyl group by Grignard coupling with methylmagnesium bromide. The methyl insertion occurred with retention of configuration and resulted in formation of the natural aplysin system from **12** in just three steps. The conversion of the methylated tricyclic ether **20** to (-)-debromoaplysin (**2**) was accomplished in two steps by double bond isomerization and selective reduction. Bromination of (-)-**2** afforded (-)-aplysin (**1**).

One of the first halogenated sesquiterpenes to be isolated from marine sources was (-)-aplysin (**1**) which occurs in



1, R_1 = Br; R_2 = H
2, R_1 = R_2 = H
4, R_1 = Br; R_2 = OH



3

the sea hare, *Aplysia kurodai*, that inhabits the eastern Pacific.[2,3] It is also found in opisthobranchs which inhabit the coasts of North America.[3] These mollusks tend to accumulate aplysin and related substances in the gut along with the presumptive aplysin precursor debromoaplysin (**2**). These compounds appear to function as antifeedants which make these slow-moving, shelless creatures unpalatable to predators.[4] They may also function as antioxidants to scavenge reactive halogen which would explain the frequent co-occurrence of the unhalogenated forms.

The sesquiterpenes in *Aplysia* appear to be derived from the red algae which constitute their principal dietary component.[3] These algae, especially of the genus *Laurencia*, have been shown to be a rich source of unusual terpenoids containing both bromine and chlorine. Aplysin (**1**) appears to be a metabolite derived from the algal constituent laurinterol (**3**); bioconversion of **3** to **1** is supported by the facile acid-catalyzed cyclization of **1** observed in vitro.[5] The absolute configurations of **1** and the related alcohol aplysinol (**4**) have been determined and are as shown.[6]

These compounds, as representatives of the first class of halogenated sesquiterpenes to be discovered in marine sources, have been of synthetic interest for several years.[7] This work describes the first reported total synthesis of (-)-**1** and (-)-**2** and was developed from our interest in mixed acetals as directing and stabilizing groups for aromatic metalation reactions and from our preliminary work on the synthesis of racemic **1** and **2**.[8] This approach, that of using an optically active alcohol for the preparation of a chiral alkoxymethyl ether protecting group, is potentially applicable to the synthesis of a wide variety of optically active natural products.

Results and Discussion

The selectivity and reactivity of aromatic methoxymethyl ethers in metalation reactions support the hypothesis that the ether oxygens coordinate with the organometallic center forming a relatively stable chelate.[9]

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