Bacteriochlorophylls-d from *Chlorobium vibrioforme*: Chromatographic Separations and Structural Assignments of the Methyl Bacteriopheophorbides

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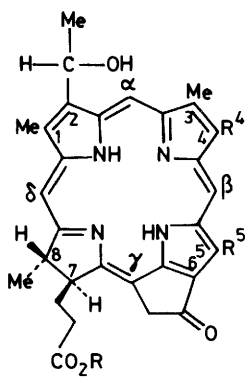
Bacteriochlorophylls-d (**2**) have been isolated from *Chlorobium vibrioforme* forma *thiosulfatophilum*, a green sulphur bacterium. Treatment of the bacteriochlorophylls with sulphuric acid in methanol affords the methyl bacteriopheophorbides (**1**) which are separated by high performance liquid chromatography (h.p.l.c.) into six major homologous fractions differing in the nature of 4-(ethyl, n-propyl, isobutyl) and 5- (methyl, ethyl) substituents. Using a combination of h.p.l.c., n.m.r., and *X*-ray crystallography, the 4-ethyl and 4-n-propyl homologues (**1a**), (**1b**), (**1c**), and (**1f**) are shown to have the (*R*) absolute stereochemistry at the 2-(1-hydroxyethyl) substituent while the 4-isobutyl compounds (**1c**) and (**1g**) are shown to have the opposite (*S*) chirality at this position. Two new methyl bacteriopheophorbides-d bearing a 4-neopentyl substituent (**1d**) and (**1h**) are isolated and characterized; these appear to be the first natural products with a neopentyl substituent to be isolated and are also assigned the (*S*) absolute stereochemistry at the 2-position on the basis of n.m.r., h.p.l.c., and *X*-ray structural evidence. The methyl bacteriopheophorbides-d from a new strain of green sulphur bacteria [*Chlorobium vibrioforme* (B1-20)] have also been studied; initially, these bacteria, which possess an abnormally short wavelength absorption in living cells, appeared to be producing major amounts of only two methyl bacteriopheophorbides-d (**1a**) and (**1e**), but after they had grown continually for more than a year, the pigment composition began to contain larger quantities of more highly methylated homologues. The bacteriopheophorbides with R^4 = Et and R^4 = Bu^i again had the (*R*) and (*S*) absolute stereochemistries, respectively, at the 2-position, but the chirality at the 2-(1-hydroxyethyl) group in the homologues with R^4 = Pr^n is shown to be a mixture of both (*R*) and (*S*), this situation resembling that in the bacteriopheophorbides-c.

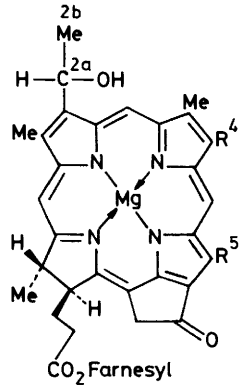
Holt and co-workers isolated six homologous methyl bacteriopheophorbides (Bmphs) (**1**) or 'Bands' from *Chlorobium thiosulfatophilum* (Strain L) by liquid–liquid partition chromatography between ether and hydrochloric acid on Celite.[1–3] The parent magnesium complexes esterified with farnesol or 'Chlorobium chlorophylls (650)' were subsequently renamed the bacteriochlorophylls-d (Bchls-d) (**2**).[4] Treatment of the Bchls-d with methanolic sulphuric acid affords the methyl bacteriopheophorbides (Bmphs-d), while brief treatment with mild acid produces the bacteriopheofarnesins, (**3**), in which the magnesium has been removed but the original esterifying alcohol (farnesol)[5] is still present on the propionate side chain. The pigments occur as series of homologues differing in the alkyl side chains at C-4 and C-5. The structures of Holt's six bands were primarily determined by identification of the maleimides produced by chromic acid oxidation of the Bmphs. MacDonald and co-workers subsequently confirmed the order of the pyrrole rings by synthesis of the pyrroporphyrins corresponding to those obtained by degradation of Bands 1—5.[6] The structure of Band 6 was confirmed by conversion into methyl pyropheophorbide-a (**4**).

Brockmann's examination of *Chlorobium vibrioforme* forma *thiosulfatophilum* revealed the same six homologous Bmphs-d as those characterized by Holt.[7] The relative abundance of the pigments, however, differed. Brockmann found that chromic acid oxidation of the homologous mixture of Bmphs-d gave, as one of the products, (2*S*,3*S*)-dihydrohaematinimide. This arises from ring D of the Bmphs and establishes the (7*S*,8*S*) configuration of these two chiral centres.[8] The configuration at the 2a chiral centre was also confirmed as (*R*) on the basis of degradative studies[8] and a Horeau analysis of the homologous Bmph-d mixture.[9]

The principal difference between the Bmphs-d and the Bmphs-c is the δ-*meso*-methyl substituent in the latter.[10–12]



(**1**) R = Me
(**3**) R = Farnesyl



(**2**)

	R^4	R^5	Holt Band Number[1–3]	Configuration at C-2a
a;	Et	Et	4	*R*
b;	Pr^n	Et	2	*R*
c;	Bu^i	Et	1	*S*
d;	Neopentyl	Et		*S*
e;	Et	Me	6	*R*
f;	Pr^n	Me	5	*R*
g;	Bu^i	Me	3	*S*
h;	Neopentyl	Me		*S*

The difference between Bands 1 and 2 and Bands 3 and 4 in the Bchls-c lies not in the nature of the *meso* substituent, but rather in the configuration at the 2a chiral centre.[13–16] The chirality at 2a shifts from (*R*) to (*S*) as the size of the alkyl moiety at R^4 increases. Thus Bmph-c [Et,Et] (Band 5) is exclusively (*R*),

Bmph-c [Pr^n,Et] (Bands 3 and 4) is an (*R*,*S*) mixture, while Bmph-c [Bu^i,Et] (Band 1) is almost completely (*S*). Brockmann had claimed that the configuration at 2a was exclusively (*R*).[9,17] It was apparent then that, according to his analytical methods, as much as 10% (Bands 1 and 3) of the (2*S*) Bmphs-c could go undetected in the homologous mixture. We therefore decided to see whether the Bmphs-d also consisted of a mixture of (*R*) and (*S*) diastereoisomers.

Isolation of the Methyl Bacteriopheophorbides-d.—*Chlorobium vibrioforme* forma *thiosulfatophilum* (Strain Lascelles 8327, DSM 263), an anaerobic green sulphur bacterium, was grown in cultures under carbon dioxide at 34 °C. The cells were harvested by centrifugation and a crude mixture of Bchls-d and carotenoids was obtained. This was treated with sulphuric acid in methanol which served to demetallate the chlorin and transesterify the 7-propionic ester to give the crude methyl bacteriopheophorbides, (Bmph-d) (**1**). The crude Bmph-d mixture was then chromatographed on neutral alumina to remove carotenoids and most of the untransesterified bacteriopheofarnesins (**3**). Careful chromatography gave pure Bmphs-d (**1**) uncontaminated by bacteriopheofarnesins (**3**). This mixture was subjected to reversed phase h.p.l.c. on a Waters 10 micron μ-Bondapack C-18 column, eluting with methanol–water or acetonitrile–water (Figure 1A). The n.m.r. spectra of Fraction 1 and Fraction 4 confirmed their identity as the Bmph-d [Et,Me] (**1e**) (Holt Band 6) and [Bu^i,Et] (**1c**) (Holt Band 1) homologues. The second and third fractions were clearly both mixtures of two homologues. This could be demonstrated by recycling. The components of Fraction 2 were Bmph-d [Et,Et] (**1a**) (Holt Band 4) and Bmph-d [Pr^n,Me] (**1f**) (Holt Band 5) while Fraction 3 contained Bmph-d [Pr^n,Et] (**1b**) (Holt Band 2) and Bmph-d [Bu^i,Me] (**1g**) (Holt Band 3). These assignments and the elution order shown in Figure 1A were subsequently confirmed by the h.p.l.c. retention times of the components of each of the homologous series R^5 = Me and R^5 = Et (Figure 1B,C). Unfortunately, even multiple recycling failed to separate the [Et,Et]/[Pr^n,Me] and [Pr^n,Et]/[Bu^i,Me] pairs satisfactorily. Consequently, the Bmphs-d were separated into two series of homologues, one bearing a methyl group at R^5 and the other an ethyl. Brockmann had already established a precedent for this separation using medium pressure liquid chromatography (m.p.l.c.) with di-isopropyl ether on silica gel.[7] We were able to obtain a similar separation on up to 1.5 g of Bmph-d crude mixture using 99:1 v/v dichloromethane–THF on silica m.p.l.c. The faster running R^5 = Et series were easily separable from the slower R^5 = Me homologues. A similar separation of the bacteriopheofarnesins-d (**3**; R^5 = Et) and (**3**; R^5 = Me) could be achieved with the same system. The ratio of pigments obtained, R^5 = Et:R^5 = Me (76:24), was identical in the Bmph-d (**1**) and bacteriopheofarnesin-d (**3**) series. Additionally, this ratio of R^5 = Et:R^5 = Me homologues of 3:1 was remarkably consistent over many batches of culture grown for periods ranging from 1—4 months.

For small-scale work, a separation into the R^5 = Et and R^5 = Me homologues with the same order of elution could be achieved by preparative t.l.c. on silica with 97:3 dichloromethane–THF as the eluant. Routine and more rapid separation of crude Bmph-d (2—3 g) into the two series in a single injection was subsequently possible using a Waters Prep 500A instrument.

The separation of each homologous series into its components by semi-preparative C-18 reverse phase h.p.l.c. was straightforward. The three major components of each series (**1a**—**c**); (**1e**—**g**) were thus obtained and characterized by n.m.r. and visible spectroscopy and mass spectrometry and found to be identical with those described by Holt and Brockmann. In order to obtain enough of each compound for elemental analysis,[13]C n.m.r. spectroscopy, and crystallization, however, it was necessary to use C-18 reverse phase h.p.l.c. on the Waters Prep 500A. In this manner several grams could be separated in a day.

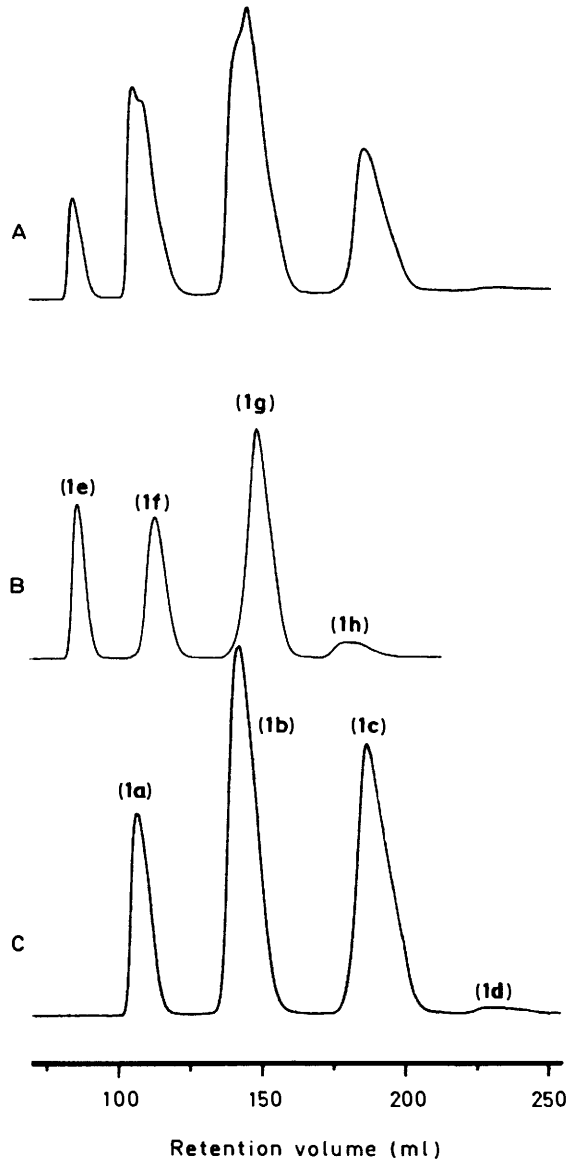


Figure 1. H.p.l.c. traces of Bmphs-d (**1**): A, complete mixture from *Chlorobium vibrioforme* forma *thiosulfatophilum*; B, the R^5 = Me series; C, the R^5 = Et series

Characterization of the Methyl Bacteriopheophorbides-d.—The spectroscopic data for Bmphs-d (**1a**—**c**) and (**1e**—**g**) agree with those reported in the literature. The mass spectra show the required parent ions at *m*/*z* 566, 580, and 594 for compounds (**1e**—**g**) and 580, 594, and 608 for (**1a**—**c**). Typical fragmentations involved dehydration (possibly thermal in origin[18]) and typical benzylic cleavages of some of the side chains.[18] Thus, compounds (**1c**) and (**1g**), where R^4 = isobutyl, show a loss of 43 a.m.u. for ($M^+ - C_3H_7$). The field desorption mass spectrum of a mixture of the R^5 = Et homologues clearly showed the three principal parent ions.

The ^{1}H n.m.r. spectra of the Bmphs-d (Table 1) are in good agreement with those reported by Brockmann.[7] The chemical shifts are concentration dependent due to aggregation, thus the

Table 1. ^{1}H N.m.r. spectra of the Bmphs-d[a]

Resonance	[Et,Et] (**1a**)	[Pr^n,Et] (**1b**)	[Bu^i,Et] (**1c**)	[neopentyl,Et] (**1d**)	[Et,Me] (**1e**)	[Pr^n,Me] (**1f**)	[Bu^i,Me] (**1g**)	[neopentyl,Me] (**1h**)	(Scheme) (**1i**)
1-Me	3.37	3.37	3.38	3.44	3.38	3.38	3.39	3.41	3.42
2a-H	6.35 (q, *J* 6.6)	6.35 (q, *J* 6.6)	6.38 (q, *J* 6.7)	6.46 (q, *J* 6.6)	6.35 (q, *J* 6.6)	6.36 (q, *J* 6.7)	6.40 (q, *J* 6.5)	6.44 (q, *J* 6.5)	6.45
2b-Me	2.10 (d, *J* 6.6)	2.11 (d, *J* 6.6)	2.13 (d, *J* 6.7)	2.17 (d, *J* 6.6)	2.10 (d, *J* 6.6)	2.11 (d, *J* 6.7)	2.14 (d, *J* 6.5)	2.16 (d, *J* 6.5)	2.16
3-Me	3.22	3.22	3.23	3.33	3.22	3.22	3.24	3.28	3.26
4a	3.67 (q, *J* 7.6)	3.64 (m)	3.54 (d, *J* 7.3)	3.68	3.63 (q, *J* 7.6)	3.60	3.53	3.61	?
4b	1.69 (t, *J* 7.6)	2.25—2.35	2.2—2.35		1.67 (t, *J* 7.5)	2.1	*d*		?
4c		1.22 (t, *J* 7.2)	1.21 (d, *J* 6.6) 1.22 (d, *J* 6.6)	1.28		1.21 (t, *J* 7.3)	1.21	1.26	1.13 (6 H ?)
5-Me					3.59	3.58	3.62	3.62	Obscured
5a	4.06 (ABX)	4.06 (ABX)	4.03 4.06 (ABX, *J* −14.3, 7.6)	4.13 (m)					
5b	1.94 (t, *J* 7.6)	1.93 (t, *J* 7.5)	1.93 (t, *J* 7.6)	1.96 (t, *J* 7.5)					
10-CH_2	5.23 5.09 (AB, *J* 19.9)	5.23 5.09 (AB, *J* 19.9)	5.22 5.09 (AB, *J* 19.9)	5.34 5.17 (AB, *J* 20.0)	5.18 5.04 (AB, *J* 19.9)	5.17 5.04 (AB, *J* 19.9)	5.20 5.07 (AB, *J* 19.9)	5.22 5.09 (AB, *J* 19.9)	5.23 5.09
7-H	4.24 (m)	4.24 (m)	4.24 (m)	4.38 (m)	4.21	4.19	4.23	4.27	4.28
7ab,- and 2a-OH	2.20—2.67	2.20—2.66	2.22—2.67 (m)	2.33—2.61 (m)	2.51—2.63 (3 H) 2.20—2.28 (2H)	2.51—2.63 (3 H) 2.14—2.27 (2 H)	2.43—2.66 (3 H) 2.19—2.30 (2 H)	2.53—2.69 2.21—2.31	*d* *d*
7d-OMe	3.62	3.62	3.62	3.65	3.62	3.62	3.62	3.64	Obscured
8-H	4.44 (m)	4.44 (m)	4.44 (m)	4.58 (m)	4.43	4.42	4.44	4.46	4.47
8-Me	1.77 (d, *J* 7.2)	1.77 (d, *J* 7.2)	1.76 (d, *J* 6.7)	1.85 (d, *J* 7.2)	1.77 (d, *J* 7.3)	1.76 (d, *J* 7.3)	1.76 (d, *J* 7.2)	1.78 (d, *J* 7.2)	1.79
α-*meso*	9.60	9.61	9.67	10.05	9.60	9.61	9.71	9.77	9.71
β-*meso*	9.49	9.48	9.47	9.83	9.39	9.36	9.42	9.52	9.43
δ-*meso*	8.48	8.48	8.48	8.76	8.48	8.48	8.50	8.63	8.51
NH	0.34 (br) −1.78 (br)	0.34 (br) −1.78 (br)	0.33 (br) −1.77 (br)	Not observed	0.26 −1.89	0.22 −1.90	0.22 −1.85	0.3 (br) −1.81	−1.81

[a] At 360 MHz, chemical shifts relative to $CHCl_3$ at 7.260 p.p.m. and coupling constants, *J* in Hz. [b] Obscured by 7d-OMe. [c] Obscured by 2b-Me, but located by decoupling. [d] Not assigned. [e] Overlapping resonances.

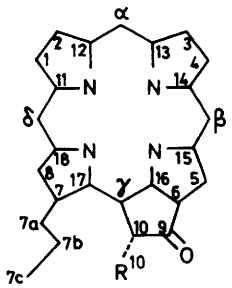
data in Table 1 were obtained at a standard concentration of 14×10^{-3}M. The R^5 = Et and R^5 = Me series can easily be distinguished by the presence of a triplet at 1.94 p.p.m. and a multiplet at *ca.* 4.06 p.p.m. for the ethyl homologues and a singlet at *ca.* 3.6 p.p.m. for the methyl homologues. The 5a-methylene group of the ethyl series is an ABX_3 system, or AB if the 5b-methyl group is decoupled. The intrinsic anisochrony of these diastereoisotopic protons may be enhanced by hindered rotation of the 5-ethyl group caused by steric interaction with the 4-alkyl side chain. Restricted rotation of the 5-ethyl group may also explain the loss of one of the signals in the ^{1}H ENDOR spectrum.[19] Within each homologous series the resonances at 4a, b, and c are diagnostic. Thus, the 4a resonance appears as a quartet at 3.67 p.p.m. for (**1a**), a multiplet at 3.64 p.p.m. for (**1b**), and a doublet at 3.54 p.p.m. for (**1c**). The 4b signal is a quartet at 1.69 p.p.m. for (**1a**) and appears as a complex multiplet in 2.2—2.35 p.p.m. region for both (**1b**) and (**1c**). This conclusion is based on integration and the effect of decoupling in this region on the 4c resonances to higher field. The 4c resonance is of course absent in compound (**1a**), appears as a triplet at 1.22 p.p.m. in (**1b**) and, characteristically, as two doublets at 1.22 and 1.21 p.p.m. in (**1c**). Other characteristic spectral features of the Bmphs-d are the one-proton quartet at 6.35 p.p.m. and three-proton doublet at 2.11 p.p.m. due to 2a-H and 2b-Me of the 2-(1-hydroxyethyl) group, and the AB quartet for the 10-CH_2 centred at 5.12 p.p.m. as well as the multiplets at 4.4 (8-H) and 4.24 p.p.m. (7-H) and the doublet at 1.77 p.p.m. (8-Me) which are diagnostic of the reduced D ring.

The assignment of the 7d-methyl ester and ring methyl resonances is based on analogy with the literature.[7,16,20] Comparison of the methyl resonances for the set R^5 = Et and R^5 = Me suggests that the signal at 3.59—3.64 p.p.m. is due to the 5-Me in the latter. The assignment of the 7d-methyl ester to the signal at 3.62 p.p.m. is confirmed by its absence in the spectra of the bacteriopheofarnesins-d.

The *meso* protons are assigned by analogy with the literature. The δ-*meso* proton in chlorophyll derivatives (or any *meso* proton adjacent to the reduced ring in a chlorin) typically resonates *ca.* 1 p.p.m. to higher field than the α- and β-*meso* resonances due to the decreased aromatic ring current.[21] The α-*meso* proton is assigned to the lowest field of the two remaining *meso* resonances due to the expected deshielding effect of the 2-(1-hydroxyethyl) group.

The ^{13}C n.m.r. spectra of the six major Bmphs-d are

Table 2. ^{13}C N.m.r. spectra of the Bmphs-d[a]



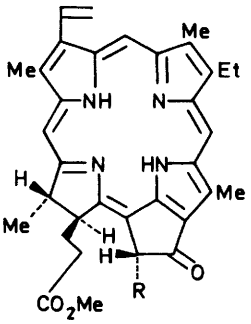
Resonance	[Et,Et] (**1a**)	[Pr^n,Et] (**1b**)	[Bu^i,Et] (**1c**)	[Et,Me] (**1e**)	[Pr^n,Me] (**1f**)	[Bu^i,Me] (**1g**)
1a	11.23	11.21(q)	11.16(q)	11.20	11.26	10.93(q)
3a		11.47(q)	11.75(q)		11.49	11.56(q)
4c		14.39(q)	23.15(q)		14.42	23.03(2 q)
5b	16.76(q)	16.75(q)	16.77(q)			
4b	17.40(q)	26.06(t)	32.03(d)	17.37(q)	26.01	31.87(d)
4a	19.45(t)	28.11(t)	35.42(t)	19.34(t)	28.11	35.18(t)
5a	20.53(t)	20.52(t)	20.51(t)	11.85(q)	11.93	11.56(q)
8a	23.05(q)	23.02(q)	23.01(q)	22.97(q)	23.03	22.70(q)
2b	25.63(q)	25.60(q)	25.68(q)	25.52(q)	25.61	25.58(q)
7b	29.77(t)	29.75(t)	29.73(t)	Obscured	Obscured	29.64(t)
7a	30.88(t)	30.97(t)	30.89(t)	30.87(t)	30.88	30.87(t)
10	47.95(t)	47.93(t)	47.90(t)	47.84(t)	47.89	47.60(t)
8	49.94(d)	49.90(d)	49.87(d)	49.85(d)	49.89	49.59(d)
7	51.55 (d + q)	51.52 (d + q)	51.52 (d + q)	51.44 (d + q)	51.48	51.22 (d + q)
7d	51.65	51.66	51.69	51.68	51.69	51.69
2a	65.54(d)	65.48(d)	65.55(d)	65.39(d)	65.50	65.23(d)
δ	92.56(d)	92.50(d)	92.46(d)	92.45(d)	92.51	92.09(d)
α	97.62(d)	97.60(d)	97.91(d)	97.58(d)	97.68	98.05(d)
β	104.09(d)	104.24(d)	104.48(d)	103.79(d)	104.06	103.71(d)
γ	106.04(s)	105.97(2)	105.90(s)	105.65(s)	105.71	105.04(s)
7c	173.45(s)	173.44(s)	173.47(s)	173.50(s)	173.48	173.46(s)
9	195.61(s)	195.60(s)	195.62(s)	196.25(s)	196.23	196.28(s)
Quaternary carbons						
5	135.52	135.46	135.42	128.05	128.09	127.51
6	129.78	129.72	129.64	130.04	130.11	129.41
1	131.37	131.30	131.07	131.27	131.29	130.61
3	135.15	135.11	134.99	135.02	135.00	134.78
12	136.09	136.59	136.54	135.99	136.77	136.90
15	136.67	136.73	137.22	137.42	137.49	136.99
11	140.76	140.76	140.84	140.83	140.77	140.88
2	141.25	141.20	141.19	141.11	141.15	141.10
4	144.93	143.15	142.43	144.73	143.09	142.02
16	149.22	149.17	149.11	148.64	148.76	148.22
14	150.74	151.18	151.55	150.54	151.10	151.01
13	155.07	154.94	154.85	154.86	154.81	154.49
17	160.01	159.93	159.89	159.99	160.02	159.52
18	171.35	171.28	171.24	171.13	171.16	170.75

[a] Chemical shifts are relative to the centre of $CDCl_3$ at 77.0 p.p.m. Concentrations (M, in $CDCl_3$); (**1a**) 0.062; (**1b**) 0.050; (**1c**) 0.051; (**1e**) 0.050; (**1f**) 0.050; (**1g**) 0.22. Experimental parameters: spectrometer frequency 50.72 MHz, 16 K data points, 18 μs pulse time, 0.95 s pulse delay, 7 000 Hz sweep width, 2 ml sample volume in a 12-mm n.m.r. tube.

presented in Table 2. The spectra were obtained for 0.05—0.06M-solutions in $CDCl_3$ except for the [Bu^i,Me] homologue (0.22M). No significant effects were observed due to the increased concentration of the latter. The assignments made in Table 2 are based primarily on comparisons with the literature and by observed trends within the six compounds. Multiplicities were determined by off-resonance decoupling.

Methyl pheophorbide-a (**5**) is the standard ^{13}C reference compound for many chlorophyll-a derivatives due to the INDOR experiments of Boxer, Closs, and Katz which allowed the unambiguous assignment of most of the fourteen quaternary carbon resonances.[22] These assignments have been slightly revised by later workers; most notably the chemical shift of C-6 was revised from 161.2 to 128.3 p.p.m. A brief history of these revisions is given in the recent review of Katz and Brown.[23] The assignments of carbons directly bonded to protons had previously been made by Katz and co-workers on the basis of selective frequency off-resonance decoupling experiments.[24,25] Smith and Unsworth[26] and Wray *et al.*[27] have extended these assignments to other derivatives of chlorophyll-a. The assignments of quaternary carbons 18, 17, 13, 14, 16, 4, 1, and 6 (in order from low to high field), the four

meso carbons, as well as carbons 7, 7a—c, 8, 8a, and 9 of the Bmphs-d given in Table 2 were made directly by comparison with the reference standards.[24–27] The assignment of C-5a in the Bmph-d R^5 = Me series and C-4a and C-4b in Bmph-d [Et,Et] (**1a**) were also assigned in this manner. The assignments of carbons 2, 2a, 2b, and 12 were based on the changes observed in these resonances when the 2-vinyl group of methyl pyropheophorbide-a (**4**) was converted into the 2-(1-hydroxyethyl) group of the Bmphs-d. Thus, when the 2-vinyl group of methyl pyrophaeophorbide-a was hydrated to give (2*RS*)-Bmph-d [Et,Me] the resonances of the sp^2 2-vinyl carbons at 121 and 128 p.p.m. in the former were replaced by signals at 25.6 (q) and 65.6 (d) p.p.m., which were thus assigned to C-2b and C-2a of the latter by their multiplicities in the off-resonance decoupled spectrum and by the expected deshielding effect of the hydroxy group.[17] The change from 2-vinyl to 2-(1-hydroxyethyl) also resulted in a new quaternary carbon resonance in the 140—142 p.p.m. region. This new resonance is undoubtedly due to C-2, assigned to 135.1 p.p.m. in methyl pyropheophorbide-a. The assignments of C-2 (141.1—141.25 p.p.m.) and C-11 (140.76—140.88 p.p.m.) in the Bmphs-d might well be reversed. However, a change at C-2a/2b would not be expected to affect C-11 significantly, and its assignment is based on a best fit to C-11 of methyl pyropheophorbide-a at 140.7 p.p.m. Assuming that the conversion of 2-vinyl into 2-(1-hydroxyethyl) exerts a similar, but smaller, downfield shift of 0.7—1.6 p.p.m. two carbons removed, C-12 was assigned to the signals at 136.1—136.9 p.p.m. (Table 2). The value of 136.7—137.5 p.p.m. for C-15 was chosen on the basis of the best fit to the reference compounds methyl pyropheophorbide-a (136.9 p.p.m.) and methyl pheophorbide-a (137.2 p.p.m.). The assignments for C-2a and -2b agree well with those made by Smith *et al.* for Bmph-c [Et,Et].[15] The same deshielding of C-2 relative to methyl pyropheophorbide-a (135.1 p.p.m.) was also noted in Bmph-c [Et,Et] (142.2 p.p.m.) and (2*RS*)-Bmph-d [Et,Me] (140.9 p.p.m.). If our assumption that C-12 also experiences a smaller 0.7—1.6 p.p.m. downfield shift is correct, then the assignments of Smith *et al.* and Tacke could well be revised. Based on these revisions, C-12 experiences a downfield shift relative to methyl pyropheophorbide-a of 2.6 p.p.m. in Bmph-c [Et,Et] and 0.7 p.p.m. in (2*RS*)-Bmph-d [Et, Me].



(**4**) R = H
(**5**) R = CO_2Me

Comparisons between the R^5 = Me and R^5 = Et series of Bmphs-d (Table 2) allowed the assignment of the remaining quaternary carbons. Changes in the resonance at *ca.* 144 p.p.m. in each series due to the different R^4 alkyl substituents {144.93 p.p.m. [Et,Et], 143.15 p.p.m. [Pr^n,Et] ($\Delta\delta$ = −1.8), and 142.43 [Bu^i,Et] ($\Delta\delta$ = −0.7 p.p.m.); 144.73 p.p.m. [Et,Me], 143.09 [Pr^n,Me] ($\Delta\delta$ = −1.6 p.p.m.), and 142.02 [Bu^i,Me] ($\Delta\delta$ = −1.0 p.p.m.)} confirm its assignment as C-4. This trend was also observed in the Bmph-d series by Tacke[17] and in the Bmph-c series by Smith and co-workers.[15]

A comparison of the Bmph-d R^5 = Me and R^5 = Et series in Table 2 shows that C-5 can be assigned to the lowest field signal at 127.5—128 p.p.m. in the R^5 = Me homologues (compare methyl pyropheophorbide-a at 128.0 p.p.m.) while this resonance is *missing* in the R^5 = Et homologues. In the R^5 = Me series C-3 is assigned to the line at 134.8—135.0 p.p.m. (compare methyl pyropheophorbide-a at 135.2 p.p.m.) while in the R^5 = Et series there are now two lines at 135.4—135.5 p.p.m. and 135.0—135.15 p.p.m. The higher field set of lines were assigned to C-3 to make it conform best with the assignment of C-3 in the R^5 = Me series. The lower field set of lines were thus assigned to C-5. The large (*ca.* 8 p.p.m.) downfield shift at C-5 on replacement of methyl by ethyl at R^5 had already been noted by Tacke, both in the Bmphs-d themselves and in maleimide model compounds.[17] A possible alternative assignment, namely that one of the signals at *ca.* 135 p.p.m. is due to C-2, while the new resonance at *ca.* 140 p.p.m. is due to C-5, appears unlikely. The assignments of the aliphatic side chain carbons 4a—c and 5a, b agree with those made for Bmph-c [Et,Et] and [Pr^n,Et][15] and the Bmphs-d R^5 = Et, as well as with maleimide model compounds[17] and are consistent with the multiplicities observed in the off-resonance decoupled spectra. The observed increase in chemical shift of carbons 4a—c as the alkyl chain is lengthened is well known in n-alkanes and empirical equations have been devised to fit the data in such systems.[28] Finally, the resonances of methyl carbons 1a and 3a coincided in the case of Bmphs-d [Et,Et] and [Et,Me] and their assignment in the other cases is at present uncertain.

The visible absorption spectra of the Bmphs-d and their derivatives are diagnostic. The Bmphs-d themselves show a prominent long wavelength (Q_y) band at 662 nm in dichloromethane and a highly intense Soret band at 408 nm. The original name '*Chlorobium* chlorophyll 650' derives from the long wavelength maximum in diethyl ether solvent of the magnesium(II) complexes. Several trends deserve comment: (1) *meso* substitution generally shifts the Q_y band *ca.* 10 nm and the Soret band *ca.* 5 nm to longer wavelength; (2) insertion of a metal ion blue-shifts the Q_y band 10—15 nm while red-shifting the Soret approximately the same amount; (3) conversion of the 2-(1-hydroxyethyl) into vinyl shifts the Q_y band *ca.* 10 nm to longer wavelength, but has little effect on the Soret band, while conversion into 2-ethyl (*meso* series) shifts Q_y only about 3 nm to shorter wavelength; and (4) substituents at the 10 position on the isocyclic E ring such as methoxycarbonyl, alkoxy, or hydroxy have practically no effect on the spectrum.

Two New Bacteriochlorophylls-d.—The powerful separation afforded by reversed phase h.p.l.c. soon revealed that at least two and possibly three new Bmphs-d (and, by extension, Bchls-d) were present in our mixture. Compounds (**1d**) and (**1h**) are marked in Figure 1B and C, while compound (**1i**), which was not always present, is not shown. Compounds (**1d**) and (**1h**) bear a neopentyl substituent at the 4-position, and represent the first natural products bearing neopentyl substituents ever to be isolated and characterized. This highly surprising finding was based on mass and n.m.r. spectroscopy, and on an *X*-ray crystal structure of the [neopentyl,Et] homologue (**1d**).[29]

The mass spectrum of compound (**1h**) shows a parent ion at *m/z* 608 and a prominent fragment ion at *m/z* 551 due to loss of C_4H_9 from the neopentyl side chain. Compound (**1d**) shows similar peaks at *m/z* 622 (M^+) and 565. The n.m.r. spectra of both compounds show nine-proton singlets at 1.28 p.p.m. for the three 4c-neopentyl Me groups and a two proton singlet at 3.6—3.7 p.p.m. for the 4a-methylene group. The rest of the spectra are fully consistent with the assigned structures. The crystal structure of compound (**1d**) also fully confirmed this assignment.[29]

Our results show that the R^5 = Me series contains a greater

Table 3. *meso*-Proton chemical shifts of (2*R*) and (2*S*) Bmphs-d[a]

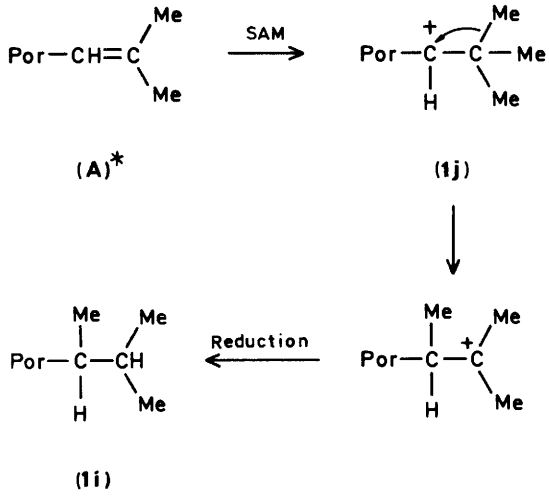
Homologue	α-(*R*)	α-(*S*)	β-(*R*)	β-(*S*)	δ-(*R*)	δ-(*S*)	Δα-β(*R*)	Δα-β(*S*)	Concentration (× 10^{-3}M)
[Et,Et]	9.560	9.582	9.460	9.456	8.467	8.458	0.100	0.126	20
[Pr^n,Et]	9.622	9.647	9.495		8.489	8.482	0.127	0.152	12
[Bu^i,Et]	9.586	9.616	9.406		8.463	8.452	0.180	0.210	26
[neopentyl,Et][b]	9.783	9.794	9.640		8.558		0.143	0.154	3
[neopentyl,Et]	10.053		9.834		8.757		0.220		
[Et,Me]	9.682	9.702	9.497		8.520	8.513	0.185	0.205	2
[Pr^n,Me]	9.705	9.724	9.503		8.529	8.522	0.202	0.221	2
[Bu^i,Me]	9.667	9.697	9.395	9.393	8.505	8.493	0.272	0.304	17
[neopentyl,Me]	9.713	9.735	9.512		8.505		0.201	0.223	≤3
[neopentyl,Me][c]		9.769	9.523		8.525			0.246	4.2

[a] Data obtained from a 1:1 racemic mixture. (2*R*), (2*S*) Assignments are based on the 1:1 racemic mixture spiked with pure natural diastereoisomer. The data for the neopentyl homologues are from mixtures racemized and then spiked with the natural diastereoisomer. [b] Measured at 50 °C; all other measurements at ambient temperature (*ca.* 22 °C). [c] Naturally occurring (2*S*) diastereoisomer.

percentage of highly alkylated homologues [(**1g**—**i**)] than the R^5 = Et series [(**1c**) and (**1d**)]. Also, the 3:1 ratio between the R^5 = Et:R^5 = Me homologues observed here is far greater than the 10:1 ratio reported by Brockmann working with the same organism. It is not clear whether Brockmann and co-workers failed to observe the small amounts of compounds (**1d**) and (**1h**) in their mixture, or whether these were absent, but it now seems clear that the proportions of alkylation can depend upon environment and growing conditions (*vide infra*).

Compound (**1i**) has not been satisfactorily characterized and was not always observed in the R^5 = Me homologous mixture. However, enough material could be obtained by preparative h.p.l.c. for an n.m.r. spectrum. This revealed that compound (**1i**) has the 2-(1-hydroxyethyl) group, three *meso* protons, a reduced ring D, and an isocyclic ring E characteristic of a Bmph-d. The 'quartet' at *ca.* 4.06 p.p.m. and triplet at 1.95 p.p.m. required for a 5-Et group are absent, thus suggesting that the R^5 substituent is in fact methyl. Impurities unfortunately obscure the 3.6—3.8 p.p.m. region, but two ring methyl groups can be clearly seen at 3.42 and 3.25 p.p.m. Since the mass spectrum gave a possible parent ion at *m/z* 608 with a prominent loss of 57 a.m.u. (C_4H_9), the substituent at C-4 probably has the empirical formula C_5H_{11}. It might be argued that compound (**1i**) is Bmph-d [neopentyl,Me] with the opposite configuration at the 2a chiral centre to compound (**1h**). This is unlikely on the basis of the chromatographic elution order, since it eluted after (2*S*)-[neopentyl,Me]; if it were (2*R*-[neopentyl,Et] it would elute before compound (**1h**). Furthermore, the high-field region of the n.m.r. spectra of compounds (**1h**) and (**1i**) are not identical. The spectrum of compound (**1i**) shows a six-proton multiplet (possibly two doublets) at 1.1 p.p.m. and two one-proton multiplets at 4.1 and 3.85 p.p.m. This is consistent with a 4-(1,2-dimethylpropyl) side chain in compound (**1i**) which could arise from Wagner–Meerwein rearrangement of an intermediate such as (**1j**) (Scheme). However, the expected three-proton doublet for the 4a-Me is not evident in the spectrum. Moreover, a loss of a C_4H_9 fragment from structure (**1i**) seems unlikely.

The Configuration of the Bmphs-d at the 2a-Chiral Centre.—Risch and Brockmann[9] used the Horeau method to define the absolute stereochemistry at the 2a-position in Bmphs-d as (*R*). The same conclusion was reached working with homologous mixtures of Bmphs-c and -e. Tacke-Karimdadian and Brockmann undertook a more rigorous proof of stereochemistry by benzoylating the 2a-hydroxy group of the homologous Bmph-d mixture from *Chlorobium vibrioforme* forma *thiosulfatophilum* (Lascelles 8327) and subjecting it to chromic acid oxidation to give maleimides. The ring A maleimide was further transformed into the benzoyl derivative of (*R*)(−)-lactic acid; the optical rotation of this material was − 11.1° at 589 nm, 20% less than the literature value of − 13.6°.[8] This could correspond to a ratio of 91:9 (*R*):(*S*) compounds in their sample. [Our results indicate that the Bmph-d mixture from the same strain *grown in Davis* contains approximately 30% of the (2*S*)-diastereoisomers.]



* (**A**) = proposed[29] biosynthetic precursor of (**1c**), (**1d**), (**1g**), and (**1h**).

Scheme. Por = Porphyrinic biosynthetic precursor; SAM = *S*-adenosylmethionine

We felt that the configuration of the 2a-centre could best be established by working with pure homologues rather than the mixture. The procedure was to racemize each of the well-characterized crystalline homologues obtained by h.p.l.c. at the 2a chiral centre by heating them in a solution of 80:20 TFA–water at 55 °C for 1 h. The electronic absorption spectra and chromatographic behaviour of the racemized Bmphs-d were identical with those of the unracemized starting material. The reversed phase h.p.l.c., however, showed that two compounds in approximately equal amounts were present in the racemized material. The n.m.r. spectra also showed the presence of two diastereoisomers, as evidenced by the doubling of many resonances, particularly the 2a-methine proton, the α- and δ-*meso* protons, and the 10-CH_2 resonances. These transformations are illustrated in Figure 2 for Bmph-d [Et,Et] (**1a**). Figure 2A shows the *meso* proton region of the n.m.r. spectrum and the h.p.l.c. tracing for the pure, naturally occurring diastereoisomer.

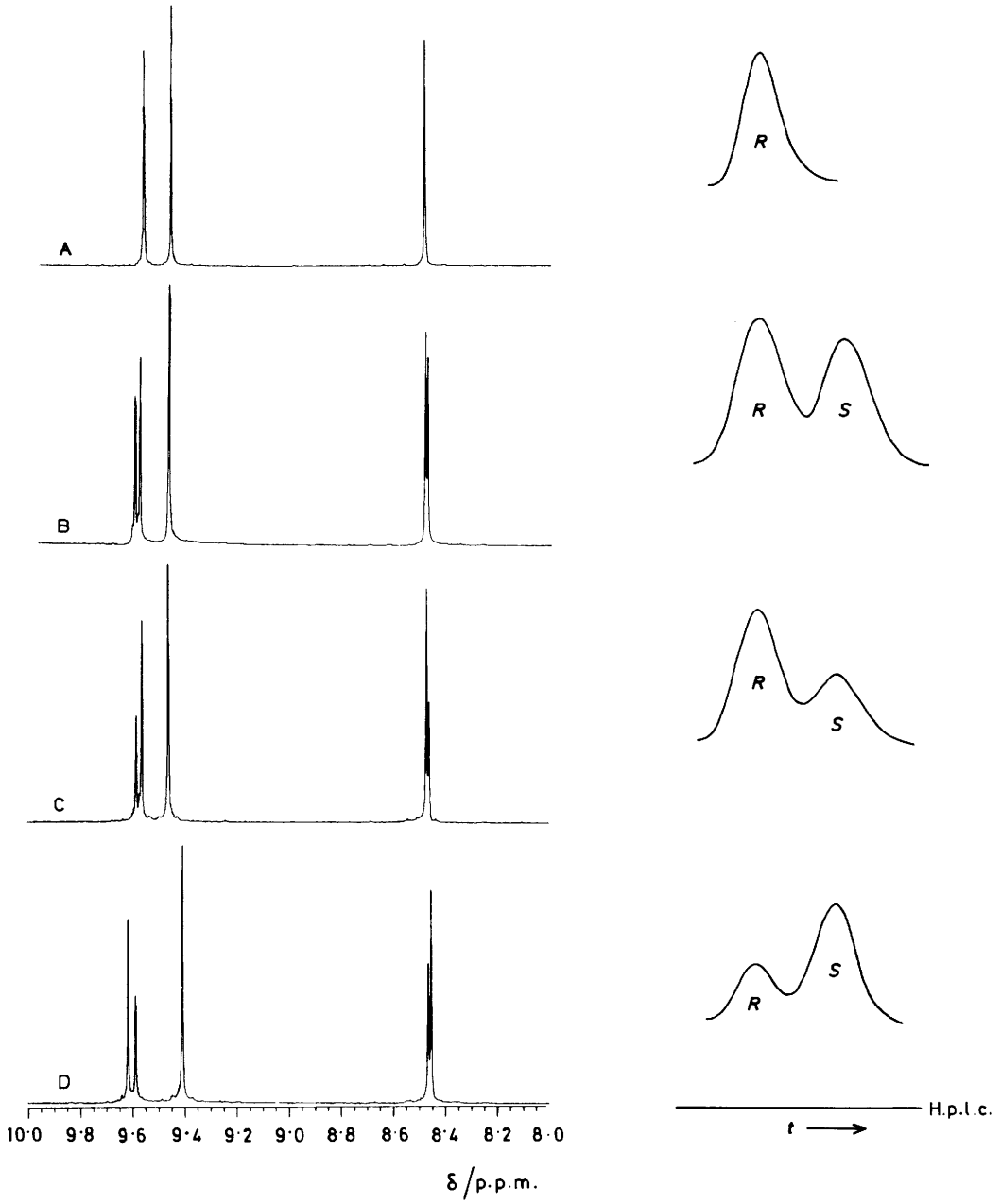


Figure 2. 360 MHz N.m.r. spectra, in $CDCl_3$ (left) and h.p.l.c. traces (right) of: A, pure Bmph-d [Et,Et] (**1a**); B, Bmph-d [Et,Et] after racemization at the 2-(1-hydroxyethyl); C, Bmph-d [Et,Et] after racemization and spiking with pure Bmph-d [Et,Et] (**1a**); D, Bmph-d [Bu^i,Et] (**1c**) after similar racemization and spiking with natural (**1c**)

Figure 2B shows the corresponding data for the mixture of diastereoisomers obtained from the treatment with aqueous TFA. Finally, in Figure 2C the diastereoisomeric mixture has been spiked with some of the natural diastereoisomer. Note the increase in intensity of the *highest* field α-*meso* resonance and the *lowest* field δ-*meso* resonance. These may be assigned to the natural diastereoisomer. The h.p.l.c. tracing now shows an increase in the size of the more mobile diastereoisomer. Results similar to these were obtained for Bmph-d [Pr^n,Et] (**1b**). However, when Bmph-d [Bu^i,Et] (**1c**) was racemized and spiked with the natural diastereoisomer, the results in Figure 2D were obtained. Here it is evident that the *lowest* field α- and *highest* field δ-*meso* resonances in the n.m.r. spectrum belong to the natural diastereoisomer. Similarly, the h.p.l.c. tracing shows that the natural diastereoisomer is now the *least* mobile. These results are exactly opposite to those obtained for the [Et,Et] and [Pr^n,Et] homologues and lead at once to the conclusion that the [Et,Et] and [Pr^n,Et] homologues possess one configuration at the 2a-chiral centre, while the [Bu^i,Et] homologue possesses the other. This conclusion was verified by *X*-ray crystallographic analysis of the [Et,Et] and [Bu^i,Et] homologues.[30] As one might expect, the [Et,Et] homologue bears the (*R*) configuration and the [Bu^i,Et] homologue the (*S*) configuration at the 2a centre. One can generalize the h.p.l.c. and n.m.r. results as follows: (1) the (*R*) diastereoisomers have smaller retention times on reversed phase h.p.l.c. than the (*S*) diastereoisomers (recycling required); (2) the α-*meso* resonance of the (*R*) diastereoisomer of a diastereoisomeric pair is to

higher field than the (*S*) diastereoisomer; conversely, the δ-*meso* proton resonance of the (*R*) diastereoisomer is to *lower* field than that of the (*S*) diastereoisomer.

It is possible to assign the chirality of a Bmph-d on the basis of the chemical shift difference between the α-and β-*meso* proton resonances. This is summarized in Table 3. The chemical shift difference between α- and β-*meso* proton resonances is consistently larger for the (*S*) diastereoisomer within each pair. For example, $\Delta\delta_{a,b}$ = 0.126 p.p.m. for (2*S*)-[Et,Et] and 0.100 p.p.m. for (2*R*)-[Et,Et]. Furthermore, the $\Delta\delta_{a,b}$ increases for both (*R*) and (*S*) diastereoisomers as the bulk of R^4 increases; for example, $\Delta\delta_{a,b}$ (2*S*)-[Pr^n,Et] = 0.152 p.p.m., while $\Delta\delta_{a,b}$ (2*R*)-[Pr^n,Et] = 0.127 p.p.m. Finally, the $\Delta\delta_{a,b}$ value also depends on the R^5 substituent; thus the $\Delta\delta_{a,b}$ for (2*S*)-[Et,Me] > $\Delta\delta_{a,b}$ (2*S*)-[Et,Et] and $\Delta\delta_{a,b}$ (2*R*)-[Et,Me] > $\Delta\delta_{a,b}$ (2*R*)-[Et,Et].

These generalizations hold for the R^5 = Me series as well, but are not shown. Both of the new Bmphs-d [neopentyl,Et] (**1d**) and [neopentyl,Me] (**1h**) have been assigned the (2*S*) configuration based on an *X*-ray crystal structure,[29] n.m.r. spectroscopy and h.p.l.c. in the first case and by n.m.r. and h.p.l.c. in the second. The [neopentyl,Et] homologue (**1d**) was racemized under standard conditions, then spiked with unracemized material and subjected to h.p.l.c. with recycling as described above for compound (**1a**). The n.m.r. spectrum of the racemized material did not show any *meso* splitting at 23 °C. However, at this temperature the *meso* proton resonances of both neopentyl homologues were noticeably broadened. Heating racemized compound (**1d**) to 50 °C split the α-*meso* proton resonance into two approximately equal peaks at 9.794 and 9.783 p.p.m. When the sample was spiked with the natural diastereoisomer, the height of the lower field of the two α-*meso* resonances increased noticeably. This again is in agreement with the trend observed for the (2*S*) diastereoisomers (Table 3). However, the chemical shift of all the *meso* protons was temperature-dependent, showing an upfield shift of *ca.* 0.2 p.p.m. on going from 23 to 50 °C.

Similar racemization and spiking experiments were performed on Bmph-d [neopentyl,Me]. The h.p.l.c. and n.m.r. results again led to a (2*S*) configurational assignment for this compound. The configurational assignments of all the Bmphs-d are given in structures (**1**)—(**3**).

Bacteriochlorophylls from a New Strain of Chlorobium vibrioforme.—In July, 1982, Professor Norbert Pfennig sent a new Bchl-d producing organism to our laboratory. This *Chlorobium vibrioforme* (B1-20) strain was isolated in Abijan, Ivory Coast, and showed an unusually low whole-cell long-wavelength visible absorption at 714 nm (typical values for *in vivo* Bchl-d range from 725—736 nm[31]). This strain was grown and harvested in Davis under the same conditions used for *Chlorobium vibrioforme* forma *thiosulfatophilum*. The crude mixture of Bchls and carotenoids was treated with 3% sulphuric acid in methanol, then chromatographed using silica t.l.c., eluting with 97:3 dichloromethane–THF. As usual, a small untransesterified bacteriopheofarnesin band was observed, as well as two Bmph bands. It was noted, however, that the mass ratio of the more mobile (R^5 = Et) Bmph band to the less mobile (R^5 = Me) band was *ca.* 20:1 as compared with the 3:1 ratio observed with *Chlorobium vibrioforme* forma *thiosulfatophilum*. The visible spectra of both Bmph bands were identical (658, 404 nm; methanol) and very close to that of Bmph-d previously obtained (660, 406 nm). The n.m.r. and mass spectra of the individual homologues subsequently obtained by h.p.l.c. further confirmed that the newly isolated *Chlorobium vibrioforme* (B1-20) was indeed a Bchl-d producing organism.

H.p.l.c. comparison of the two newly obtained Bmph-d bands with the R^5 = Et and R^5 = Me homologous mixtures

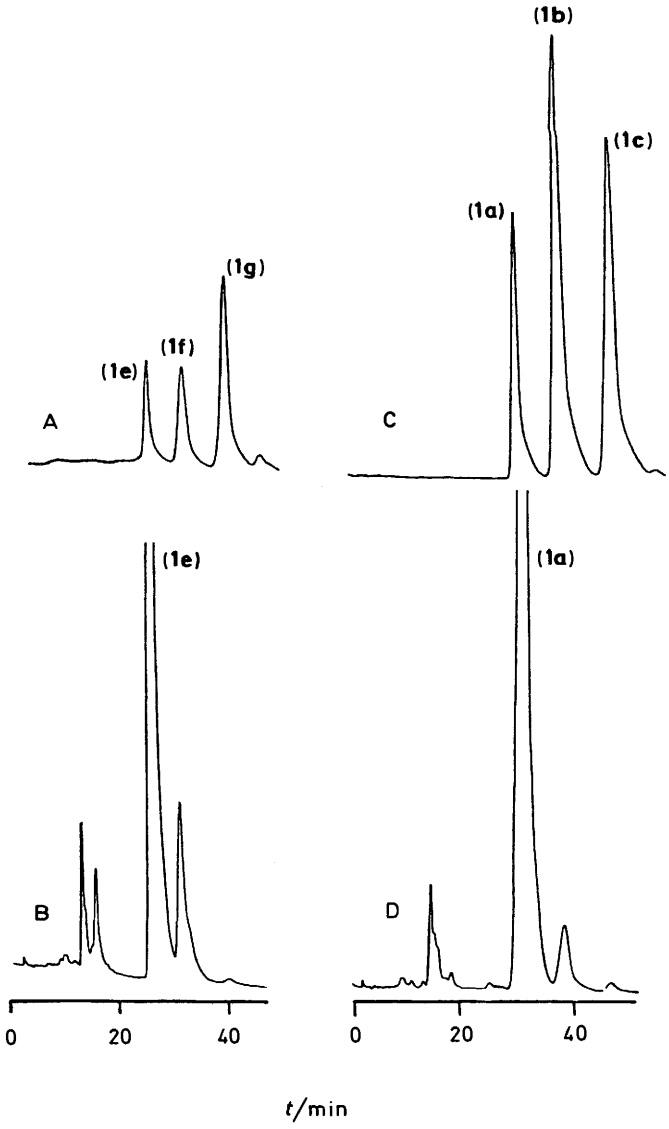


Figure 3. H.p.l.c. comparisons of the Bmphs-d from *C. vibrioforme* forma *thiosulfatophilum* and *C. vibrioforme* (B1-20). A, Bmph-d R^5 = Me from *C. vibrioforme* forma *thiosulfatophilum*; B, Bmph-d R^5 = Me from *C. vibrioforme* (B1-20); C, Bmph-d R^5 = Et from *C. vibrioforme* forma *thiosulfatophilum*; D, Bmph-d R^5 = Et from *C. vibrioforme* (B1-20). Conditions: C-18 RP, 7.8 × 250 mm, 10 micron, 85:15 MeOH–H_2O, 2.6 ml/min, detector at 660 nm

previously obtained from *C. vibrioforme* forma *thiosulfatophilum* confirmed that the more mobile band from the new Ivory Coast strain indeed consisted of the R^5 = Et homologues, while the less mobile minor band contained the R^5 = Me homologues. These comparisons are shown in Figure 3. It was immediately evident that the major constituent of the Ivory Coast strain was Bmph-d [Et,Et] (**1a**) (84% by mass) along with smaller amounts of the [Pr^n,Et] (*ca.* 8%) and [Bu^i,Et] (*ca.* 3%) homologues. In the R^5 = Me series, the only compound which could be positively identified was the [Et,Me] homologue (**1e**) (5.5% by mass of the total mixture). These results obviously contrast sharply with those obtained with *C. vibrioforme* forma *thiosulfatophilum*. The relative amounts are based on semi-preparative h.p.l.c. of the Ivory Coast strain. The mass and n.m.r. spectra of each of the purified homologues agree with the assignments made (see Experimental section).

The stereochemistry at 2a of the Bmphs-d from the new Ivory

Coast strain was also investigated by n.m.r. spectroscopy. The [Et,Et] (**1a**) and [Et,Me] (**1e**) homologues from the new strain showed only single α- and β-*meso* proton resonances, before and after spiking with the corresponding pure diastereoisomers from *C. vibrioforme* forma *thiosulfatophilum*. The appropriate control experiments were run on the racemized mixture of each homologue from *C. vibrioforme* forma *thiosulfatophilum* and showed that the splitting of these resonances could be clearly observed under the experimental conditions. Thus the [Et,Et] and [Et,Me] homologues from *C. vibrioforme* (B1-20) have the (*R*) configuration at 2a. The [Bu^i,Et] homologue from the new strain showed only single *meso* proton resonances. When this homologue was added to a mixture of Bmph-d [Bu^i,Et] from *C. vibrioforme* forma *thiosulfatophilum* racemized at 2a, the intensity of the lower field α-*meso* proton resonance [(2*S*) diastereoisomer] relative to the higher field α-*meso* proton resonance [(2*R*) diastereoisomer] increased from 1.7:1 to 1.9:1, leading to the assignment of the (2*S*) configuration to this homologue. Finally, the [Pr^n,Et] homologue from the Ivory Coast strain was found to occur 'naturally' as a *ca.* 1:1 (*R*,*S*) mixture at the 2a chiral centre, this somewhat resembling the situation for the [Pr^n,Et] homologue in the Bchl-c.[13,14] This was also confirmed by h.p.l.c. recycle. Attempts to characterize

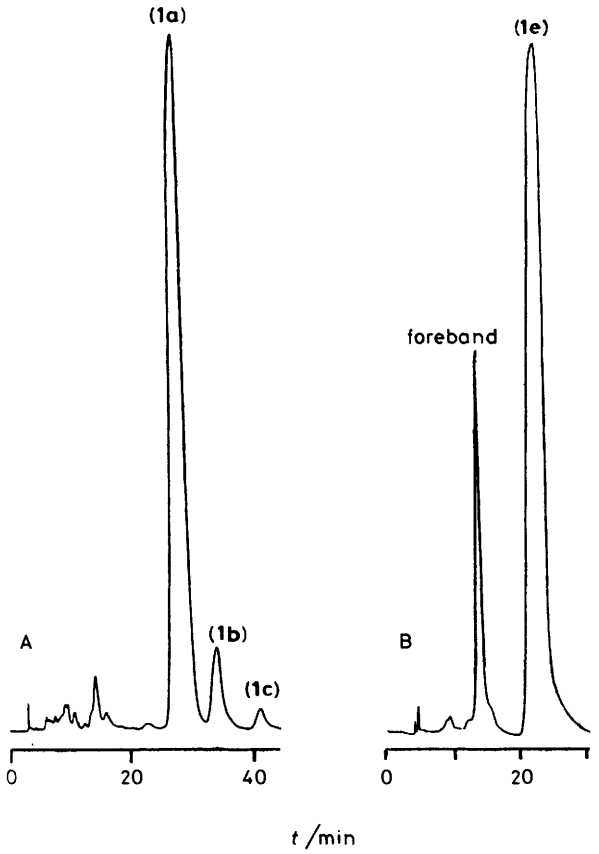


Figure 4. Semi-preparative h.p.l.c. separations of the Bmphs-d from *C. vibrioforme* (B1-20). A, R^5 = Et; B, R^5 = Me. Conditions as for Figure 3

the fore-band observed in the semi-preparative h.p.l.c. (Figure 4) of the R^5 = Me series from the Ivory Coast strain were unsuccessful. [The visible spectrum of this material was similar to a Bmph-d (662, 410 nm, dichloromethane) as was the n.m.r. spectrum. There were three *meso* protons, with the α and β split into pairs of resonances. The usual sharp quartet for the 2a-methine proton was replaced by two broad multiplets in roughly the same region. The h.p.l.c. characteristics of the material were unaltered after treatment with diazomethane, suggesting that it is not an ester hydrolysis product.]

Some 14 months later, after the Ivory Coast strain had been grown continuously in our laboratory, the Bchls-d were again isolated (Experimental section). H.p.l.c. of the Bchls indicated the presence of more homologues than had been observed earlier for the Bmphs-d of the same strain (*vide supra*). In particular, Bchl-d [Bu^i,Et] (**2c**) was a major homologue and this clearly indicated that the isomeric composition of the Bchls-d produced by *C. vibrioforme* (B1-20) was changing. To verify this, the crude Bchl-d mixture left over from h.p.l.c. was treated with 5% sulphuric acid in methanol to give the Bmphs-d. This mixture was purified by silica t.l.c. The major R^5 = Et band was compared by h.p.l.c. (Figure 5) with the Bmphs-d isolated

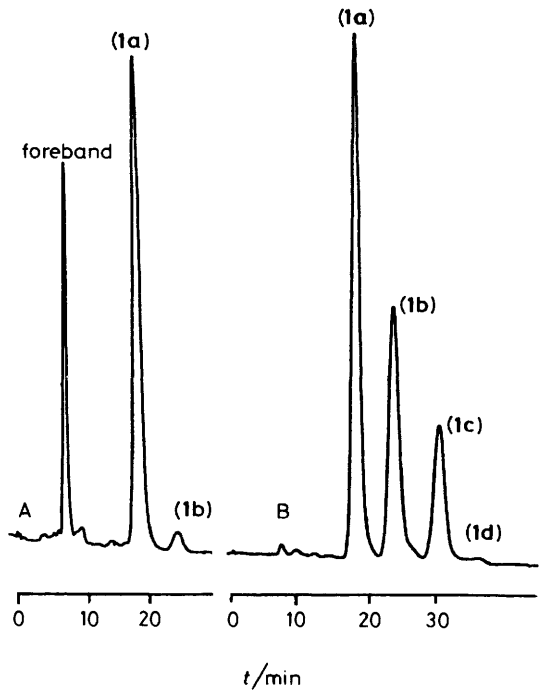


Figure 5. H.p.l.c. traces of the Bmph-d R^5 = Et produced by *C. vibrioforme* (B1-20). A, harvested on January 10, 1983; B, harvested on March 17, 1984. Conditions: C-18 RP, 10 micron, Waters Z-Module, 85:15 MeOH–H_2O, 2.5 ml/min, detector at 660 nm

from the same strain 14 months earlier. This comparison showed that the composition of the homologous mixture had definitely changed with time; the Ivory Coast strain was now producing markedly larger amounts of the more highly methylated C-4 homologues. This is to be compared with the mixtures of Bchls-d and -c produced by *C. vibrioforme* forma *thiosulfatophilum* and *Prosthecochloris aestuarii*, respectively, whose pigment composition has hardly changed over a period of more than 15 years.

Experimental

M.p.s, which are uncorrected, were measured on a Thomas/Bristoline microscopic hot-stage apparatus. Electronic absorption spectra were measured on a Hewlett-Packard 8450A spectrophotometer using solutions in dichloromethane, and mass spectra were measured on a Finnigan 3200 spectrometer (direct insertion probe, 70 eV, 50 μA, source temperature from

200 to 300 °C). High resolution mass spectra were run at the Department of Pharmaceutical Chemistry, University of California, San Francisco. ^{1}H N.m.r. spectra were obtained at 360 MHz on a Nicolet NT-360 spectrometer, or at 500 MHz on a Nicolet NT-500 spectrometer. ^{13}C N.m.r. spectra were obtained at 50 MHz on a Nicolet NT-200 spectrometer. The chemical shifts are reported relative to $CHCl_3$ at 7.260 p.p.m. (360 and 500 MHz). The phrase 'dried and evaporated' means drying with sodium sulphate, followed by evacuation with a Büchi rotary evaporator under house or oil pump vacuum. Elemental analyses were determined by the Microanalytical Laboratory at the University of California, Berkeley.

Monitoring of reactions by thin-layer chromatography (t.l.c.) was performed on cut strips (approx. 2 cm × 6 cm) of E. Merck silicagel 60 F254 precoated (0.25-mm thickness) plastic-backed sheets. Preparative t.l.c. was performed on freshly prepared 20 cm × 20 cm t.l.c. plates of *ca.* 1-mm thick E. Merck silicagel GF 254 and 60 G. Plates were activated prior to use by heating at 150 °C for at least 8 h. Two types of packing material were employed in column chromatography; E. Merck neutral alumina (70—230 mesh) and Merck silicagel 60. The alumina was deactivated with either 6% H_2O (Brockmann Grade III) or 15% H_2O (Brockmann Grade V) before use. A 250-ml J. T. Baker column was used for flash chromatography. M.p.l.c. utilized an FMI Model RPG150 pump, an Altex rotary injection valve, and a 1 000-mm Altex glass chromatography column. Analytical h.p.l.c. was performed on a Waters Associates HPLC instrument equipped with a Model 6000A solvent delivery system, a Valco model C6U injector and a Perkin-Elmer LC55B variable wavelength detector. A Waters stainless steel semi-preparative μBondapak C-18 column (reverse phase, 7.8 × 250 mm, 10 μm particle size) or the Waters Z-Module system equipped with a 10-μm C-18 reversed phase cartridge were used. The solvent systems used are specified where appropriate. All solvents were reagent (not h.p.l.c.) grade and were filtered through a 0.45-μm Millipore filter before use. The house de-ionized water was distilled before use. Preparative h.p.l.c. was performed on a Waters Prep-500A chromatograph using either C-18 reversed phase or silica cartridges. An ISCO Model 1840 variable wavelength absorbance monitor was used for detection. The samples, but not the solvents, were filtered through 0.45-μm filters before injection.

Culture and Harvesting of Chlorobium vibrioforme.—*Chlorobium vibrioforme* forma *thiosulfatophilum* Strain Lascelles 8327, provided by Professor Norbert Pfennig, University of Konstanz, was grown in either 4.6-l or 20-l containers. The growth medium for a 20-l batch is given in Table 4; the amounts were scaled down proportionately for the 4.6-l batches. The cells were grown under fluorescent light with magnetic stirring at a constant room temperature of about 30 °C. The containers were subcultured under CO_2 and sealed to prevent unnecessary exposure to air. No attempt was made to regulate the pH of the medium or to maintain sterile conditions. When the system was in full production, four 20-l carboys and three 4.6-l flasks were in use. At each harvest three of the 20-l carboys and two of the 4.6-l flasks were centrifuged. The remaining 20-l carboy was used to subculture a new 20-l carboy; similarly, a new 4.6-l flask was subcultured from the remaining one. The subcultures were performed as follows: a new carboy was filled with 20 l of fresh growth medium (Table 4). This was bubbled with $CO_{2(g)}$ for *ca.* 5 min. Then 9-l of this solution was transferred under pressure of CO_2 to a third carboy, followed by transfer under CO_2 of 9 l of dark green bacterial culture into the new carboy. The 9 l of fresh medium transferred to the third carboy was then used to replenish the old culture. A similar procedure was followed for the 4.6-l cultures, using 1.5 l of old culture to subculture a new batch. It was found that when subculture volumes of 1—4 l (rather than 9 l) were used to subculture the 20-l carboys, the bacteria frequently aggregated at the bottom of the carboy. This was reflected in relatively poor yields of Bmphs-d for some batches.

Table 4. Yields of Bmphs-d (crude) from *Chlorobium vibrioforme*[a]

Harvest	Age (days)[b]	Vol (l)	Yield of Bmphs-d (mg)	(mg/l)
1	7	20	257	12.9
2	4.5	17	215	12.6
3	5	80	387	4.8
4	6—7	140	504	4.8
5	6	80	264	3.3
6	19	9.2	156	17.0
7	10	49.2	714	14.5
8	10	49.2	575	11.7
9	10	49.2	630	12.8
10	10	49.2	678	13.8
11	10	49.2	880	17.9
12	10	49.2	890	18.1
13	17	69.2	1 872	27.0
14	*31*	49.2	1 388	28.2
15	*28*	69.2	914	13.2
16	*74*	69.2	1 392	20.1
17	*64*	69.2	1 523	22.0
18	*48*	69.2	1 267	18.3
19	*51*	69.2	1 110	16.0
20	*109*	69.2	1 590	23.0
21	*99*	69.2	1 400	20.2
22	*155*	73.8	1 530	20.7
		1 318	20.126×10^3	15.3

[a] For comparison, Brockmann[8] reports a yield of 810 mg of Bmph-d from 50 l of culture, or 16.2 mg/l. [b] Values in italics are weighted average ages.

The cells were harvested at intervals ranging from 10 days to 5 months by centrifugation on a Sharples AS-16 centrifuge. The wet cell mass was scraped and rinsed out of the centrifuge rotor with the aid of methanol. The solid clumps were well macerated with a spatula, dissolved in methanol, and transferred to a 2-l Erlenmeyer flask. The resulting bright green solution was filtered through Whatman No. 1 paper on a Buchner funnel. The residual cell debris was rinsed with methanol and discarded. The methanolic extract was evaporated to near dryness on the rotary evaporator and then diluted with a large volume of dichloromethane. This solution was cooled in the refrigerator for several hours to reduce emulsion problems, then divided into several portions and rinsed with saturated, aqueous sodium chloride. Occasionally, severe emulsions resulted, but these could usually be broken by filtration of the emulsified material through a glass wool plug. The organic phase was dried (Na_2SO_4) and evaporated. All these operations and subsequent chromatography were conducted in dim light.

The resulting crude mixture of Bchls-d (**2**) and carotenoids was then treated with 95:5 v/v methanol–concentrated sulphuric acid (typically 500 ml for 69.2 l harvest) for 4 h at room temperature under nitrogen. The reaction mixture was then divided into two portions. Each portion was diluted with an equal volume of dichloromethane and rinsed with one-half volume of water (*i.e.* 250:250:125 methanol–dichloromethane–water). The organic phase was then rinsed with saturated aqueous sodium hydrogen carbonate, dried and evaporated to give the crude Bmphs-d.

Residual bacteriopheofarnesins-d (**3**) and carotenoids were removed by column chromatography on Grade III neutral

alumina (*ca.* 200—230 g/1.5 g crude Bmph-d mixture). Elution typically progressed from toluene, to 50:50 toluene–dichloromethane, to 80:20 dichloromethane–toluene (complete removal of yellow and orange material), and then 100% dichloromethane, whereupon the bacteriopheofarnesins-d (**3**) eluted. The Bmphs-d (**2**) could be washed down when desired with 1% methanol in dichloromethane as a dark purple band. Later this procedure was shortened considerably by using the same system with the 250-ml flash chromatography column. Yields are given in Table 4. The homologous Bmph-d mixture was then subjected to h.p.l.c. or preparative h.p.l.c. on the Waters Model Prep 500A. In either case, the eluant was 99:1 dichloromethane–THF on silica gel. On occasion, as much as 1.4 g of the homologous Bmph-d mixture could be resolved into the R^5 = Et and R^5 = Me series on the 15 × 1 000-mm m.p.l.c. column, although 0.8—1.0 g gave a more consistent separation. The separation took *ca.* 8 h.

In the case of preparative h.p.l.c., 1—1.6 g of Bmph-d mixture dissolved in dichloromethane (10 ml) was injected onto one silica cartridge. With a flow rate of 150 ml/min a good separation into the R^5 = Et and R^5 = Me series was achieved in 2.5 h using 20 l of solvent.

In either system, the chromatographic elution order was the same: traces of bacteriopheofarnesins-d (two bands), followed by the Bmphs-d R^5 = Et, then the Bmphs-d R^5 = Me. On a smaller scale, separation of the two series was performed on preparative silica t.l.c. plates, eluting with 97:3 dichloromethane–THF.

Separation of the individual homologues from either series was accomplished by semi-preparative or preparative C-18 reversed phase chromatography. On a 7.8 × 250 mm semi-preparative column, eluting with 85:15 v/v methanol–water, 1—2 mg of mixture injected in 100—500 μl of methanol could be separated in 1 h. The fractions were collected in round-bottom flasks and the solvent simply evaporated under oil-pump vacuum. The residues were dissolved in dichloromethane, transferred to vials, and dried under a stream of nitrogen. In some cases, a white residue was observed in the flasks, probably due to leaching of the C-18 packing from the column. This and other non-chlorin impurities could be substantially removed, if necessary, by passing the h.p.l.c. fraction, in dichloromethane, through a short plug of dichloromethane-washed Grade III alumina packed in a Pasteur pipet. The column was eluted thoroughly with dichloromethane to remove non-polar impurities, then with 1% methanol in dichloromethane to remove the Bmph-d.

Yields and relative amounts of the Bmphs-d in each series obtained from semi-preparative h.p.l.c. are given in Table 5. Given the small masses involved, particularly for the minor homologues, the possibility of non-chlorin contaminants, and the fact that the Bmphs were present as dried films, not crystalline solids these figures should be regarded as approximate.

Injections of 1—3.5 g of the R^5 = Et and R^5 = Me series were made on the Prep 500A, using one or two C-18 cartridges, depending on the amount of material. A run typically took *ca.* 3.5 h and used *ca.* 24 l of 85:15 v/v methanol–water at a flow rate of 150 ml/min. The chromatography was monitored by the visible absorbance at 650 nm. The general appearance of the chromatograms was as expected from the analytical h.p.l.c. traces. However, due to the large amount of material injected, the separation between the peaks was hardly baseline, nor was this desirable, and multiple fractions were collected. The purity of each fraction was determined by analytical h.p.l.c. on a 7.8 × 250-mm C-18 column and the fractions were combined accordingly. The fractions which contained >90% of a single homologue were recrystallized directly, while the rest were combined and re-injected. Thus many different fractions and crystal batches were obtained, which made the direct determination of relative yields difficult.

Table 5. Yields of Bmphs-d from semi-preparative h.p.l.c. of *Chlorobium vibrioforme*[a]

Sample	[Et,Me] (%)	[Pr^n,Me] (%)	[Bu^i,Me] (%)	[neopentyl,Me] (%)	(**1i**) (%)
1	3.5 (22)	5.0 (32)	7.3 (46)		
2	3.6 (14)	8.2 (32)	13.7 (53)	0.2 (1)	
3	8.5 (14)	15.6 (27)	29.9 (51)	2.9 (5)	1.9(3)

Sample	[Et,Et] (%)	[Pr^n,Et] (%)	[Bu^i,Et] (%)	[neopentyl,Et] (%)
1	1.8 (21)	4.7 (55)	2.0 (24)	
2	3.7 (26)	6.6 (47)	3.7 (25)	0.3 (2)

[a] Mass in mg.

Several other points should be mentioned: (1) the Bmphs-d are not very soluble in methanol, therefore they were usually dissolved in 10—30 ml of acetone for injection. Even then it was sometimes difficult to get the desired amount of Bmph-d into solution in a reasonable volume. (2) When the recycling feature of the Prep 500A was used on the initial injection of homologous mixture, solvent volumes of up to 40 l were required due to peak broadening. We generally found it preferable to collect a large number of fractions on the first run, examine and combine these fractions as appropriate, and then re-inject. Thus for the R^5 = Et series four separations would typically be required: (A) initial mixture of R^5 = Et homologues, (B) a mixture of the [Et,Et] and [Pr^n,Et] homologues, (C) a mixture of the [Pr^n,Et] and [Bu^i,Et] homologues, and (D) a mixture of the [Bu^i,Et] and [neopentyl,Et] homologues. (3) Since it was tedious to evaporate the large volumes of methanol–water from the reverse-phase preparative h.p.l.c., we usually diluted the fractions with distilled water and extracted with dichloromethane. The pigments were thus readily obtained in a small volume of organic solvent.

Analytical Data.—(For ^{1}H n.m.r. data see Table 1; for ^{13}C n.m.r. data see Table 2.) *Bmph-d* [Et,Et], (**1a**), crystallized from CH_2Cl_2–n-hexane, m.p. 215—216 °C (Found: C, 72.1: H, 7.0; N, 9.5. $C_{35}H_{40}N_4O_4$ requires C, 72.39; H, 6.94; N, 9.65%); $\lambda_{max.}(CH_2Cl_2)$ 660 (ε 6.00 × 10^4), 604 (1.02 × 10^4), 536 (1.23 × 10^4), 504 (1.19 × 10^4), 472 (4.50 × 10^3), and 408 nm (1.26 × 10^5); *m/z* 580 (100%, M^+), 562 (44, $M^+ - H_2O$).

Bmph-d [Pr^n,Et] (**1b**), crystallized from CH_2Cl_2–n-hexane, m.p. 183 °C (Found: C, 72.8; H, 7.2; N, 9.4. $C_{36}H_{42}N_4O_4$ requires C, 72.70; H, 7.12; N, 9.42%); $\lambda_{max.}(CH_2Cl_2)$ 660 (ε 5.98 × 10^4), 604 (9.87 × 10^3), 536 (1.20 × 10^4), 504 (1.15 × 10^4), and 408 nm (1.27 × 10^5); *m/z* 594 (100%, M^+), 576 (22, $M^+ - H_2O$).

Bmph-d [Bu^i,Et] (**1c**), crystallized from CH_2Cl_2–n-hexane, m.p. 155—157 °C (Found: C, 73.1; H, 7.3; N, 9.2. $C_{37}H_{44}N_4O_4$ requires C, 73.00; H, 7.28; N, 9.20%); $\lambda_{max.}(CH_2Cl_2)$ 660 (ε 6.27 × 10^4), 604 (1.07 × 10^4), 536 (1.28 × 10^4), 504 (1.24 × 10^4), 472 (4.2 × 10^3), and 408 nm (1.33 × 10^5); *m/z* 608 (100%, M^+), 590 (65, $M^+ - H_2O$).

Bmph-d [neopentyl,Et] (**1d**), crystallized from methanol, m.p. 181 °C, $\lambda_{max.}(CH_2Cl_2)$ (relative absorbance) 662 (0.524), 606 (0.121), 536 (0.136), 506 (0.141), and 410 nm (1.38). High resolution mass spectrum: 622.342 31 ($C_{38}H_{46}N_4O_4$ requires *M*, 622.351 92); 565.279 14 ($M^+ - C_4H_9$ requires 565.281 49).

Bmph-d [Et,Me] (**1e**), crystallized from CH_2Cl_2–methanol, m.p. 236—238 °C (Found: C, 70.4; H, 6.7; N, 9.5. $C_{34}H_{38}N_4O_4$

requires C, 72.06; H, 6.76; N, 9.89%. $C_{34}H_{38}N_4O_4 \cdot CH_3OH$ requires C, 70.21; H, 7.07; N, 9.36%); $\lambda_{max.}(CH_2Cl_2)$ 660 (ε 5.81 × 10^4), 604 (9.57 × 10^3), 536 (1.13 × 10^4), 504 (1.15 × 10^4), 472 (4.40 × 10^3), and 408 nm (1.24 × 10^5); *m/z* 566 (100%, M^+), 548 (100, $M^+ - H_2O$).

Bmph-d [Pr^n,Me] (**1f**), crystallized from CH_2Cl_2–n-hexane, m.p. 205 °C (Found: C, 72.3; H, 6.95; N, 9.6. $C_{35}H_{40}N_4O_4$ requires C, 72.39; H, 6.94; N, 9.65%); $\lambda_{max.}(CH_2Cl_2)$ 662 (ε 5.86 × 10^4), 604 (9.54 × 10^3), 536 (1.13 × 10^4), 504 (1.14 × 10^4), and 408 nm (1.23 × 10^5); *m/z* 580 (100, M^+).

Bmph-d [Bu^i,Me] (**1g**), crystallized from CH_2Cl_2–n-hexane, m.p. 209 °C (Found: C, 72.45; H, 7.15; N, 9.3. $C_{36}H_{42}N_4O_4$ requires C, 72.70; H, 7.12; N, 9.42%); $\lambda_{max.}(CH_2Cl_2)$ 662 nm (ε 6.01 × 10^4), 604 (9.56 × 10^3), 536 (1.13 × 10^4), 504 (1.16 × 10^4), and 408 nm (1.29 × 10^5); *m/z* 594 (100%, M^+), 576 (48.5, $M^+ - H_2O$), 551 (49), 534 (16), and 533 (21).

Bmph-d [neopentyl,Me] (**1h**), precipitated from CH_2Cl_2 with hexane, a solid, m.p. 217—218 °C, $\lambda_{max.}(CH_2Cl_2)$ 662 (ε 6.54 × 10^4), 604 (1.06 × 10^4), 536 (1.21 × 10^4), 504 (1.30 × 10^4), 472 (5.20 × 10^3), and 408 nm (1.29 × 10^5); *m/z* 608 (44.5, M^+), 590 (44, $M^+ - H_2O$), 551 (96, $M^+ - C_4H_9$), and 535 (100). High resolution mass spectrum: 608.331 81 ($C_{37}H_{44}N_4O_4$ requires 608.336 26); 551.259 60 ($M^+ - C_4H_9$ requires 551.265 84).

General Procedure for the Racemization of Bmphs-d.—The general procedure is illustrated for Bmph-d [Bu^i,Et] (**1c**). Crystalline (**1c**) (10.3 mg) was placed in a two-neck 25-ml round-bottom flask equipped with stir bar and reflux condenser. Then a solution of H_2O (2 ml) diluted to 10 ml with TFA was added. The flask was cooled to 0 °C on an ice-bath and nitrogen was bubbled vigorously into the solution *via* a needle for 10 min. The flask was covered with aluminium foil to exclude light, the ice-bath was removed, and the reaction mixture was heated in an oil bath at 55 °C bath temperature with stirring under nitrogen for 1 h. The reaction mixture was then poured into H_2O (20 ml) and shaken with dichloromethane (35 ml). The organic layer was removed and the aqueous layer extracted with dichloromethane until colourless. The combined organic layers were carefully neutralized with saturated aqueous sodium hydrogen carbonate (35 ml), dried and evaporated. The crude product was immediately treated with excess of ethereal diazomethane, even though significant baseline due to 7-propionic methyl ester hydrolysis was not usually observed. T.l.c. (95:5 dichloromethane–THF) showed two spots: one of them, R_F 0.44, co-spots with the starting material. The other, lesser, spot had R_F 0.60 and was probably the 2-(1,1,1-trifluoroacetoxy)-Bmph-d [Bu^i,Et]. The crude product was chromatographed on a 1 × 22 cm column of Grade III neutral alumina. The faster running fraction (0.6 mg) was eluted with dichloromethane, then the racemized (**1c**) was rapidly eluted with 98:2 dichloromethane–methanol. The yield of racemized compound (**1c**) was 7.0 mg (68%).

Analytical Data for Racemized Bmphs-d from Chlorobium vibrioforme *forma* thiosulfatophilum (*Lascelles* 8327).—(2*RS*)*Bmph-d* [Et,Et], [(2*RS*)-(**1a**)]. Compound (**1a**) (8.8 mg) gave the [(2*RS*)-(**1a**)] (5.8 mg, 66%) mixture, plus a small amount of the faster-running by-product.

[(2RS)-(**1a**)], $\lambda_{max.}(CH_2Cl_2)$ (relative absorbance) 660 (48), 604 (8.2), 536 (9.9), 504 (9.5), 472 (3.6), and 408 nm (100); δ (360 MHz; $CDCl_3$) (A) [(2*RS*)-(**1a**)] (5.8 mg, 20 × 10^{-3}M) 9.623, 9.604 [2 s, α-*meso*-H, (2*RS*), integral 1.00:1.12], 9.493 (s, β-*meso*-H), 8.458, 8.476 [2 s, δ-*meso*-H, (2*RS*)], 6.33—6.37 [2 q, 2a-H, (2*RS*)], 5.04—5.26 [2 AB q, 10-CH_2, (2*RS*)], 4.44 (m, 8-H), 4.24 (m, 7-H), 4.06 (ABX_3, 5a-CH_2), 3.68 (q, 4a-CH_2), 3.622, 3.615 [2 s, 7d-OMe, (2*RS*)], 3.377, 3.365 [2 s, 1-Me, (2*RS*)], 3.22 (s, 3-Me), 2.20—2.69 (m, 7a,b-CH_2CH_2–), 2.10 (d, *J* 6.6 Hz, 2b-Me), 1.93 (t, *J* 7.5 Hz, 5b-Me), 1.780, 1.760 [2 d, *J* 6.1 Hz, 8-Me, (2*RS*)], 1.69 (t, *J* 7.6 Hz, 4b-Me), 0.34, −1.77 (2 br s, NH). (B) [(2*RS*)-(**1a**)] spiked with the natural diastereoisomer (**1a**) (2.4 mg). Major diastereoisomer [naturally occurring (2*R*)-(**1a**)] 9.560 (s, α-*meso*-H, relative integral 2.21), 8.467 (s, δ-*meso*-H), 6.304 (q, *J* 6.7 Hz, 2a-H), 5.206, 5.069 (AB q, 10-CH_2), 3.620 (s, 7d-OMe), 3.351 (s, 1-Me), and 1.764 (d, *J* 7.2 Hz, 8-Me). Minor diastereoisomer [(2*S*)-(**1a**)] 9.582 (s, α-*meso*-H, relative integral 1.00), 8.458 (s, δ-*meso*-H), 6.269 (q, *J* 6.7 Hz, 2a-H), 5.187, 5.056 (AB q, 10-CH_2), 3.630 (s, 7d-OMe), 3.340 (s, 1-Me), and 1.74 (d, partially obscured, 8-Me). Common resonances: 9.460 (s, β-*meso*-H), 4.42 (m, 8-H), 4.21 (m, 7-H), 4.04 (ABX_3, 5a-CH_2), 3.66 (q, 4a-CH_2), 3.205 (s, 3-Me), 2.20—2.65 (m, 7a,b-CH_2CH_2–), 2.08 (d, 2b-Me), 1.92 (t, 5b-Me), and 1.68 (t, 4b-Me).

Faster-running by-product, $\lambda_{max.}(CH_2Cl_2)$ (relative absorbance) 660 (49), 604 (8.8), 536 (10.5), 504 (10), 472 (4), and 408 nm (100); δ (360 MHz; $CDCl_3$) 9.77 (s, α-*meso*-H), 9.56 (s, β-*meso*-H), 8.50 (s, δ-*meso*-H), 5.93 (q, 2a-H); 5.27, 5.12 (AB q, 10-CH_2), 4.47 (m, 8-H), 4.29 (m, 7-H), 4.10 (q, 5a-Me), 3.71 (q, 4a-CH_2), 3.60 (s, 7d-OMe), 3.37 (s, 1-Me), 3.26 (s, 3-Me), 2.2—2.7 (m, 7a,b-CH_2CH_2–), 2.107, 2.000 (2 d, *J* 6.6 Hz, 2b-Me), 1.95 (t, 5b-Me), 1.79 (d, 8-Me), 1.71 (t, 4b-Me), and −1.67 (NH).

(2RS)-*Bmph-d* [Pr^n,Et] [(2RS)-(**1b**)] Compound (**1b**) (9.7 mg) gave [(2*RS*)-(**1b**)] (3.5 mg, 36%) mixture, plus *ca.* 2 mg of the faster-running by-product.

[(2*RS*)-(**1b**)], $\lambda_{max.}(CH_2Cl_2)$ (relative absorbance) 660 (47), 604 (8), 536 (9.7), 504 (9.4), 474 (3.6), and 408 nm (100); δ (360 MHz; $CDCl_3$) (A) [(2*RS*)-(**1b**)] (3.5 mg, 12 × 10^{-3}M) 9.674, 9.656 [2 s, α-*meso*-H, (2*RS*), integral 1.00:1.16]; 9.516 (s, β-*meso*-H), 8.501, 8.495 [2 s, δ-*meso*-H, (2*RS*)], 6.39—6.43 [2 q, overlapping, 2a-H, (2*RS*)], 5.07—5.28 [2 AB q, 10-CH_2, (2*RS*)], 4.46 (m, 8-H), 4.27 (m, 7-H), 4.08 (ABX_3, 5a-CH_2), 3.66 (t, *J* 7.3 Hz, 4a-CH_2), 3.617, 3.612 [2 s, 7d-OMe, (2*RS*)], 3.402, 3.394 [2 s, 1-Me, (2*RS*)], 3.244 (s, 3-Me), 2.18—2.60 (m, 7a,b-CH_2CH_2–), 2.14 (d, *J* 6.5 Hz, 2b-Me), 1.94 (t, *J* 7.6 Hz, 5b-Me), 1.788, 1.774 [2 d, *J* 7.0 Hz, 8-Me, (2*RS*)], 1.24 [two unresolved t, 4c-Me, (2*RS*)]. (B) [(2*RS*)-(**1b**)] spiked with the natural diastereoisomer (**1b**) (1.0 mg). Major diastereoisomer [[2*R*)-(**1b**)] 9.622 (s, α-*meso*-H, relative integral 2.24), 8.489 (s, δ-*meso*-H), 6.370 (q, *J* 6.7 Hz, 2a-H), 5.234, 5.093 (AB q, 10-CH_2), 3.613 (s, 7d-OMe), 3.380 (s, 1-Me), 2.116 (d, *J* 6.7 Hz, 2b-Me), 1.781 (d, *J* 7.3 Hz, 8-Me). Minor diastereoisomer [(2*S*)-(**1b**)] 9.646 (s, α-*meso*-H, relative integral 1.00), 8.482 (s, δ-*meso*-H), 6.360 (q, *J* 6.7 Hz, 2a-H), 5.223, 5.087 (AB q, 10-CH_2), 3.619 (s, 7d-OMe), 3.375 (s, 1-Me), 2.121 (d, *J* 6.5 Hz, 2b-Me), and 1.765 (d, *J* 7.2 Hz, 8-Me). Common resonances 9.494 (s, β-*meso*-H), 4.45 (m, 8-H), 4.26 (m, 7-H), 4.07 (ABX_3, 5a-CH_2), *ca.* 3.6 (obscured, 4a-CH_2), 3.23 (s, 3-Me), 2.21—2.66 (m, 7a,b-CH_2CH_2–), 1.93 (t, 5b-Me), and 1.22 (t, *J* 7.3 Hz, 4c-Me).

Faster-running by-product, $\lambda_{max.}(CH_2Cl_2)$ (relative absorbance) 660 (47), 604 (8.3), 536 (10), 504 (9.4), 472 (3.8), and 408 nm (100); δ (360 MHz; $CDCl_3$) 9.77 (s, α-*meso*-H), 9.54 (s, β-*meso*-H), 8.50 (s, δ-*meso*-H), 5.93 (q, *J* 6.7 Hz, 2a-H), 5.26, 5.12 (AB q, 10-CH_2), 4.47 (m, 8-H), 4.29 (m, 7-H), 4.10 (ABX_3, 5a-CH_2), 3.65—3.72 (m, 4a-CH_2, plus a possible impurity), 3.60 (s, 7d-OMe), 3.37 (s, 1-Me), 3.26 (s, 3-Me), 2.65—2.75 (1 H), 2.5—2.6 (1 H), 2.2—2.4 (2 H) (7a,b-CH_2CH_2–), 2.12—2.2 (m, 2 H, 4b-CH_2), 2.110, 2.102 (2 d, 2b-Me), 1.95 (t, *J* 7.6 Hz, 5b-Me), 1.79 (d, *J* 7.2 Hz, 8-Me), 1.23 (t, *J* 7.2 Hz, 4c-Me), and 0.48, −1.66 (2 br s, NH).

(2RS)-*Bmph-d* [Bu^i,Et] [(2RS)-(**1c**)]. $\lambda_{max.}(CH_2Cl_2)$ (relative absorbance) 660 (33, 604 (8), 536 (9.7), 504 (9.4), 472 (3.6), and 408 nm (100); δ (360 MHz; $CDCl_3$) (A) [(2*RS*)-(**1c**)] (8.05 mg, 26 × 10^{-3}M) 9.673, 9.652 [2 s, α-*meso*-H, (2*RS*), relative integral 1.00:0.75], 9.482 (s, β-*meso*-H), 8.494, 8.487 [2 s, δ-*meso*-H, (2*RS*)], 6.40 (m, 2a-H), 5.06—5.27 [2 AB q, 10-CH_2, (2*RS*)], 4.45 (m, 8-H), 4.25 (m, 7-H), 4.06 (ABX_3, 5a-CH_2), 3.620, 3.615 [2 s, 7d-OMe, (2*RS*)], 3.54 (d, *J* 7.3 Hz, 4a-CH_2), 3.395, 3.387 [2 s, 1-Me, (2*RS*)], 3.235 (s, 3-Me), 2.22—2.64 (m, 7a,b-CH_2CH_2–),

2.13 (d, *J* 6.6 Hz, 2b-Me), 1.93 (t, *J* 7.6 Hz, 5b-Me), 1.779, 1.764 [2 d, *J* 7.0 Hz, 8-Me, (2*RS*)], and 1.21, 1.23 (2 d, 4c-Me). (B) [(2*RS*)-(**1c**)] spiked with the natural diastereoisomer (**1c**) (2.3 mg). Major diastereoisomer [naturally occurring (2*S*)-(**1c**)] 9.616 (s, α-*meso*-H, relative integral 1.84), 8.452 (s, δ-*meso*-H), 6.300 (q, *J* 6.7 Hz, 2a-H), 5.177, 5.051 (AB q, 10-CH_2), 3.633 (s, 7d-OMe), 3.346 (s, 1-Me), 2.100 (d, *J* 6.7 Hz, 2b-Me), and 1.735 (d, *J* 7.2 Hz, 8-Me). Minor diastereoisomer [(2*R*)-(**1c**)] 9.586 (s, α-*meso*-H, relative integral 1.00), 8.463 (s, δ-*meso*-H), 6.331 (q, *J* 6.7 Hz, 2a-H), 5.196, 5.064 (AB q, 10-CH_2), 3.625 (s, 7d-OMe), 3.360 (s, 1-Me), 2.092 (d, *J* 6.5 Hz, 2b-Me), and 1.75 (d, partially obscured, 8-Me). Common resonances 9.406 (s, β-*meso*-H), 4.42 (m, 8-H), 4.20 (m, 7-H), 4.02 (ABX_3, 5a-CH_2), 3.50 (d, *J* 7.3 Hz, 4a-CH_2), 3.20 (s, 3-Me), 2.22—2.65 (m, 7a,b-CH_2CH_2–), 1.91 (t, *J* 7.7 Hz, 5b-Me), and 1.212, 1.206 (2 d, *J* 6.5 Hz, 4c-Me).

Faster-running by-product, $\lambda_{max.}$(CH_2Cl_2) (relative absorbance) 660 (45.5), 604 (8), 536 (9.3), 504 (9.4), 474 (3.7), and 408 nm (100).

(2RS)-*Bmph-d* [neopentyl,Et] [(2RS)-(**1d**)]. Compound (**1d**) (*ca.* 1 mg) was racemized. [(2*RS*)-(**1d**), $\lambda_{max.}$(CH_2Cl_2) (relative absorbance) 666 (37.3), 606 (7.4), 560 (4.3), 538 (8.2), 506 (8.6), and 412 nm (100); δ (360 MHz; $CDCl_3$; 50 °C), see Table 3.

(2RS)-*Bmph-d* [Et,Me] [(2RS)-(**1e**)]. Compound (**1e**) (*ca.* 1.8 mg) gave [(2*RS*)-(**1e**)] (0.5 mg), plus a small amount of the faster running by-product. δ (360 MHz; $CDCl_3$) (A) [(2*RS*)-(**1e**) (0.5 mg; 2 × 10^{-3}M) 9.702, 9.682 [2 s, α-*meso*-H, (2*RS*), relative integral 1.00:1.04], 9.497 (s, β-*meso*-H), 8.520, 8.513 [2 s, δ-*meso*-H, (2*RS*)], 6.42—6.45 [2 q, 2a-H, (2*RS*)], 5.06—5.27 [2 AB q, 10-CH_2, (2*RS*)], 4.47 (m, 8-H), 4.27 (m, 7-H), 3.69 (q, *J* 7.6 Hz, 4a-CH_2), 3.66 (s, 5-Me), 3.614, 3.608 [2 s, 7d-OMe, (2*RS*)], 3.416, 3.408 [2 s, 1-Me, (2*RS*)], 3.26 (s, 3-Me), 2.15 (d, *J* 6.7 Hz, 2b-Me), 1.803, 1.787 [2 d, *J* 7.0, (2*RS*)], and 1.70 (t, *J* 7.7 Hz, 4b-Me). (B) [(2*RS*)-(**1e**)] spiked with the natural diastereoisomer (**1e**) (*ca.* 1.8 mg). Major diastereoisomer [naturally occurring (2*R*)-(**1e**)] 9.637 (s, α-*meso*-H, relative integral 4.38), 8.498 (s, δ-*meso*-H), 6.389 (q, *J* 6.7 Hz, 2a-H), 5.20 , 5.07 (AB q, 10-CH_2), 3.392 (s, 1-Me), 2.122 (d, *J* 6.7 Hz, 2b-Me), and 1.785 (d, *J* 7.2 Hz, 8-Me). Minor diastereoisomer [(2*S*)-(**1e**)] 9.662 (s, α-*meso*-H, relative integral 1.00), 8.487 (s, δ-*meso*-H), 6.364 (q, *J* 6.7 Hz, 2a-H), 5.18, 5.05 (AB q, 10-CH_2), 3.382 (s, 1-Me), 2.11 (d, partially obscured, 2b-Me), and 1.765 (d, partially obscured, 8-Me). Common resonances 9.432 (s, β-*meso*-H), 4.44 (m, 8-H), 4.22 (m, 7-H), 3.66 (q, *J* 6.7 Hz, 4a-CH_2), 3.62 (s, 7d-OMe and 5-Me), 2.2—2.7 (m, 7a,b-CH_2CH_2–), and 1.68 (t, *J* 7.5 Hz, 4b-Me).

(2RS)-*Bmph-d* [Pr^n,Me] [(2RS)-(**1f**)]. Compound (**1f**) (*ca.* 2 mg) gave [(2*RS*)-(**1f**)] (0.7 mg) plus a small amount of the faster-running by-product. δ (360 MHz; $CDCl_3$) (A) [(2*RS*)-(**1f**)] (0.7 mg, 2 × 10^{-3}M) 9.724, 9.705 [2 s, α-*meso*-H, (2*RS*), relative integral 1.00:0.98], 9.503 (s, β-*meso*-H), 8.528, 8.522 [2 s, δ-*meso*-H, (2*RS*)], 6.43—6.46 [2 q, 2a-H, (2*RS*)], 5.07—5.28 [2 AB q, 10-CH_2, (2*RS*)], 4.47 (m, 8-H), 4.27 (m, 7-H), 3.66 (t, partially obscured, 4a-CH_2), 3.67 (s, 5-Me), 3.614, 3.609 [2 s, 7d-OMe, (2*RS*)], 3.421, 3.413 [2 s, 1-Me, (2*RS*)], 3.26 (s, 3-Me), 2.2—2.7 (m, 7a,b-CH_2CH_2–), 2.128, 1.56 [2 d, *J* 6.6 Hz, 2b-Me, (2*RS*)], 1.789, 1.803 [2 d, *J* 7.1 Hz, 8-Me, (2*RS*)], and 1.22 (t, 4c-Me). (B) [(2*RS*)-(**1f**)] spiked with the natural diastereoisomer (**1f**). Major diastereoisomer [naturally occurring (2*R*)-(**1f**)], 9.617 (s, α-*meso*-H, relative integral 3.68), 8.479 (s, δ-*meso*-H), 6.372 (q, *J* 6.7 Hz, 2a-H), 5.18, 5.05 (AB, q, 10-CH_2), 3.619 (s, 5-Me), 3.587 (s, 7d-OMe), 3.380 (s, 1-Me), 1.768 (d, *J* 7.4 Hz, 8-Me), and 1.255 (t, *J* 7.2 Hz, 4c-Me). Minor diastereoisomer, [(2*S*)-(**1f**)] 9.649 (s, α-*meso*, relative integral 1.00), 8.466 (s, δ-*meso*), 6.344 (q, *J* 6.7 Hz, 2a-H), 5.15, 5.03 (AB q, 10-CH_2), 3.629 (s, 5-Me), 3.609 (s, 7d-OMe), 3.371 (s, 1-Me), 1.75 (d, partially obscured, 8-Me), and 1.209 (t, 4c-Me). Common resonances: 9.373 (s, β-*meso*-H), 4.42 (m, 8-H), 4.21 (m, 7-H), 3.22 (s, 3-Me), 2.15—2.7 (m, 7a,b-CH_2CH_2–), and 2.11 (d, *J* 6.8 Hz, 2b-Me).

(2RS)-*Bmph-d* [Bu^i,Me] [(2RS)-(**1g**)]. $\lambda_{max.}$(CH_2Cl_2) (relative absorbance) 662 (46.5), 604 (7.4), 536 (8.7), 504 (14.3), 472 (3.3), and 408 nm (100); δ (360 MHz; $CDCl_3$) (A) [(2*RS*)-(**1g**)] (4.4 mg, 15 × 10^{-3}M) 9.698, 9.668 [2 s, α-*meso*-H, (2*RS*), relative integral 1.00:1.05], 9.393 (s, β-*meso*-H), 8.505, 8.493 (2 s, δ-*meso*-H, (2*RS*)], 6.37—6.41 (2 q, 2a-H, (2*RS*)], 5.02—5.24 [2 AB q, 10-CH_2, (2*RS*)], 4.44 (m, 8-H), 4.22 (m, 7-H), 3.629, 3.620 (2 s, 7d-OMe), 3.60 (s, 5-Me), 3.51 (d, 4a-CH_2), 3.396, 3.385 [2 s, 1-Me, (2*RS*)], 3.23 (s, 3-Me), 2.2—2.7 (m, 7a,b-CH_2CH_2–), 2.13 (d, 2b-Me), 1.75, 1.77 [2 d, 8-Me, (2*RS*)], 1.20 (d, *J* 6.5 Hz, 4c-Me). (B) [(2*RS*)-(**1g**)] spiked with the natural diastereoisomer (**1g**). Major diastereoisomer [naturally occurring (2*S*)-(**1g**)] 9.658 (s, α-*meso*-H, relative integral 2.23), 8.458 (s, δ-*meso*-H), 6.325 (q, *J* 6.7 Hz, 2a-H), 5.12, 5.01 (AB q, 10-CH_2), 3.641 (s, 7d-OMe), 3.358 (s, 1-Me), 2.113 (d, *J* 6.7 Hz, 2b-Me), and 1.79 (d, *J* 7.3 Hz, 8-Me). Minor diastereoisomer [(2*R*)-(**1g**)] 9.616 (s, α-*meso*-H, relative integral 1.00), 8.475 (s, δ-*meso*-H), 6.360 (q, *J* 6.8 Hz, 2a-H), 5.15, 5.03 (AB q, 10-CH_2), 3.628 (s, 7d-OMe), 3.377 (s, 1-Me), 2.106 (d, partially obscured, 2b-Me), and 1.75 (d, partially obscured, 8-Me). Common resonances: 9.298 (s, β-*meso*), 4.40 (m, 8-H), 4.16 (m, 7-H), 3.547 (s, 5-Me), 3.47 (d, 4a-CH_2), 3.21 (s, 3-Me), 2.15—2.6 (m, 7a,b-CH_2CH_2–), and 1.193, 1.189 (2 d, *J* 6.6 Hz, 4c-Me).

Faster-running by-product, visible spectrum (CH_2Cl_2) (relative absorbance) 662 (44.8), 604 (7.8), 536 (9.1), 504 (9.3), 472 (3.7), and 408 nm (100).

(2RS)-*Bmph-d* [neopentyl, Me] [(2RS)-(**1h**)]. Compound (**1h**) (1.2 mg) was racemized, $\lambda_{max.}$(CH_2Cl_2) (relative absorbance) 664 (44.4), 606 (7.6), 554 (3.3), 536 (8.5), 504 (9.5), 472 (3.9), and 410 nm (100). δ (360 MHz; $CDCl_3$; 23 °C) (A) [(2*RS*)-(**1h**)] (< 3 × 10^{-3}M) 9.735, 9.713 [2 s, α-*meso*-H, (2*RS*), relative integral 0.55:0.38]; 9.512 (s, β-*meso*-H, relative integral 1.0), 8.505 (s, δ-*meso*, relative integral 0.80). (B) [(2*RS*)-(**1h**)] spiked with *ca.* 0.4 mg of natural diastereoisomer (**1h**): Major diastereoisomer [naturally occurring (2*S*)-(**1h**)] 9.739 (s, α-*meso*, relative integral 0.70). Minor diastereoisomer [(2*R*)-(**1h**)] 9.716 (s, α-*meso*-H, relative integral 0.28). Common resonances: 9.507 (s, β-*meso*-H, relative integral 1.00), 8.504 (s, δ-*meso*-H, relative integral 0.78).

Growth and Harvesting of Chlorobium vibrioforme (B1-20) (*Ivory Coast*).—This strain was received from Professor Norbert Pfennig (Konstanz) in July, 1982. The cultures were grown using the same growth medium and under the same conditions as those described for *C. vibrioforme* forma *thiosulfatophilum* (Lascelles 8327) in 2- or 4.6-l containers. On January 10, 1983, the first 2-l batch was harvested by decanting most of the water from the culture and adding methanol (*ca.* 600 ml) to 'precipitate' the cells (alternatively, the cells could have been centrifuged). The growing cell mass of this strain superficially resembled the Bchl-c producing *Prosthecochloris aestuarii* more than *C. vibrioforme* forma *thiosulfatophilum* in that the cells clumped together in stringy masses rather than being dispersed more or less evenly throughout the medium. The precipitated cells were then collected on a Buchner funnel and re-dissolved in methanol. The green solution was then diluted with saturated aqueous sodium chloride and dichloromethane. The pigments went into the organic layer and this was dried and evaporated. The residue was stored at 4 °C overnight and then treated with 3% sulphuric acid (50 ml) in methanol for 4 h at room temperature in the dark under nitrogen. The solution was then diluted with an equal volume of dichloromethane and rinsed successively with water and saturated aqueous sodium hydrogen carbonate. The organic layer was dried and evaporated to give the crude Bmphs-d. Chromatography on 20 × 20 cm silica t.l.c. plates, eluting with 97:3 dichloromethane–THF gave a trace of faster running green material (presumably untransesterified bacteriopheofarnesins-d) and two green Bmph-d bands. The faster running band (10

mg) was subsequently shown to be the R^5 = Et series, while the slower running band (< 1 mg) was the R^5 = Me series. The visible spectra of the two Bmph-d bands were identical [658 (0.55), 404 nm (1.13), methanol]. This was compared with a standard mixture of R^5 = Me homologues prepared from *C. vibrioforme* forma *thiosulfatophilum* [660 (0.57), 406 nm (1.36), methanol]. Each of the new Bmph-d bands was subjected to h.p.l.c. on the 7.8 × 250-mm C-18 RP column, eluting with 85:15 methanol–water at a flow rate of 2.6 ml/min. The injection volume was 25 μl. These tracings are shown in Figure 3.

On May 5, 1983, a culture of *C. vibrioforme* (B1-20) (4.6-l) was harvested and converted into the Bmphs-d according to the foregoing procedure. The crude mixture of carotenoids and Bmphs-d was then subjected to flash chromatography on Grade III neutral alumina, eluting first with toluene, then dichloromethane, then dichloromethane–methanol to remove the Bmphs-d. This improved procedure required only 15 min. The Bmphs-d were then chromatographed on silica thick layer plates, eluting with 97:3 dichloromethane–THF. This yielded 174.6 mg (94.5%) of the faster-running R^5 = Et homologues and 10.2 mg (5.5%) of the slower-running R^5 = Me homologues. Each of the series was subjected to semi-preparative h.p.l.c. on the 7.8 × 250-mm C-18 RP column, eluting with 85:15 methanol–water at a flow rate of 2.6 ml/min with an injection volume of 100 μl (see Figure 4). The yields are given in Table 6.

The spectroscopic data for the Bmphs-d thus collected are presented below.

(2R)-*Bmph-d* [Et,Et] (**1a**). *m/z* 580 (100%, M^+), 562 (49); δ (360 MHz; $CDCl_3$) 9.53 (s, α-*meso*-H), 9.43 (s, β-*meso*-H), 8.45 (s, δ-*meso*-H), 6.26 (q, 2a-H), 5.19, 5.05 (AB q, 10-CH_2), 4.41 (m, 8-H), 4.19 (m, 7-H), 4.02 (ABX_3, 5a-CH_2), 3.62 (s, 7d-OMe), 3.33 (s, 1-Me), 3.19 (s, 3-Me), 2.50—2.63 and 2.20—2.27 (2 m, 5 H, 7a,b-CH_2CH_2– and 2a-OH), 2.06 (d, *J* 6.6 Hz, 2b-Me), 1.92 (t, *J* 7.5 Hz, 5b-Me), 1.75 (d, *J* 7.2 Hz, 8-Me), 1.67 (t, *J* 7.6 Hz, 4b-Me), and 0.28, −1.84 (2 br s, NH).

(2RS)-*Bmph-d* [Pr^n,Et] (**1b**). *m/z* 594 (62.5%, M^+), 576 (47); δ (360 MHz; $CDCl_3$) 9.697, 9.679 [2 s, α-*meso*-H, (2*RS*)], 9.54 (s, β-*meso*-H), 8.51 (s, δ-*meso*-H), 6.44 (m, br, 2a-H), 5.26, 5.12 (AB q, 10-CH_2), 4.47 (m, 8-H), 4.29 (m, 7-H), 4.09 (ABX_3, 5a-CH_2), 3.61 (s, 7d-OMe), 3.42 (s, 1-Me), 3.26 (s, 3-Me), 2.52—2.71 and 2.2—2.36 (2 m, 5 H, 7a,b-CH_2CH_2– and 2a-OH), 2.171, 2.132 [2 d, *J* 6.8 Hz, 2b-Me, (2*RS*)], 1.94 (t, *J* 7.5 Hz, 5b-Me), 1.798, 1.784 [2 d, *J* 7.0 Hz, 8-Me, (2*RS*)], 1.22 (t, 4c-Me), and 0.41 −1.72 (2 br s, NH).

(2S)-*Bmph-d* [Bu^i,Et] (**1c**). *m/z* 608 (64.7, M^+), 590 (38); δ (360 MHz; $CDCl_3$) 9.71 (s, α-*meso*-H), 9.54 (s, β-*meso*-H), 8.51 (s, δ-*meso*-H), 6.45 (q, 2a-H), 5.26, 5.12 (AB q, 10-CH_2), 4.49 (m, 8-H), 4.29 (m, 7-H), 4.09 (ABX_3, 5a-CH_2), 3.85 (q, 4a-CH_2), 3.42 (s, 1-Me), 3.26 (s, 3-Me), 2.2—2.7 (m, 7a,b-CH_2CH_2–), 2.16 (d, *J* 6.9 Hz, 2b-Me), 1.94 (t, *J* 7.7 Hz, 5b-Me), 1.78 (d, *J* 7.7 Hz, 8-Me), 1.226, 1.218 (2 d, 4c-Me), and −1.71 (br s, NH).

(2R)-*Bmph-d* [Et,Me] (**1e**). *m/z* 566 (83, M^+), 548 (58); δ (360 MHz; $CDCl_3$) 9.56 (s, α-*meso*-H), 9.34 (s, β-*meso*-H), 8.46 (s, δ-*meso*-H), 6.32 (q, *J* 6.6 Hz, 2a-H), 5.15, 5.02 (AB q, 10-CH_2), 4.31 (m, 8-H), 4.17 (m, 7-H), *ca.* 3.7 (m, obscured, 4a-CH_2), 3.62 (s, 5-Me), 3.56 (s, 7d-OMe), 3.35 (s, 1-Me), 3.20 (s, 3-Me), 2.5—2.66 and 2.16—2.28 (2 m, 5 H, 7a,b-CH_2CH_2– and 2a-OH), 2.08 (d, *J* 6.6 Hz, 2b-Me), 1.76 (d, *J* 7.3 Hz, 8-Me), 1.66 (t, *J* 7.5 Hz, 4b-Me), and 0.16, −1.94 (2 br s, NH).

The basis for the configurational assignments at 2a is discussed in the text.

Preparation of Bacteriochlorophylls-d from C. vibrioforme (B1-20).—On March 17, 1984, a 1-l batch of *C. vibrioforme* (B1-20) was harvested by diluting with acetone and extracting with dichloromethane–THF. The organic layer was dried and evaporated. The residue was dissolved in *p*-dioxane and the Bchls-d were precipitated by addition of water (overnight at 4 °C). The gummy precipitate was collected on a sintered glass funnel and washed with purified n-hexane (distilled over CaH_2). This gave an easily handled solid which was dissolved directly in acetone and filtered for h.p.l.c. Several combinations of methanol–water, acetonitrile–water, and acetonitrile–THF on C-18 reversed phase h.p.l.c. were tried. The best combination, however, was 75:25 v/v acetone–water. For semi-preparative h.p.l.c. 250-μl injections were made on a Waters 10 μm C-18 RP column with the Z-Module system, eluting at 2.0 ml/min. Three fractions were collected and after the second, major, fraction had been collected, the solvent was changed to 100% acetone to rapidly elute the third fraction. The system was then re-equilibrated for *ca.* 10 min at 3.0 ml/min in 75:25 acetone–water before the next injection. The major second fraction was re-injected, using 73:27 acetone–water. This system was not ideal, and there appeared to be some loss of product due to precipitation on the column (removed with 100% acetone). Going to 30% water gave unreproducible results. The two major Bchls-d [Et,Et] (**2a**) and [Bu^i,Et] (**2b**) esterified with farnesol were collected. Only enough material for n.m.r. spectroscopy was obtained and no yield data are available. Each of these compounds was converted into the corresponding Bmphs-d by treatment with 5% sulphuric acid in methanol for 4 h. They were then diluted with dichloromethane, washed with water and saturated aqueous sodium hydrogen carbonate, dried and evaporated.

The material (*ca.* 20 mg) remaining from the h.p.l.c. of the Bchls-d from *C. vibrioforme* (B1-20) was converted into the Bmph-d mixture by treatment with 5% sulphuric acid in methanol (20 ml) in the dark under argon for 4 h, then diluted with dichloromethane, washed with water and saturated aqueous sodium hydrogen carbonate, dried and evaporated. The Bmphs-d were plated on silica, eluting with 97:3 dichloromethane–THF. Only the major band due to the R^5 = Et homologues was collected, as the R^5 = Me band appeared to be too small to work with. The R^5 = Et fraction was compared (Figure 5) with the Bmphs-d R^5 = Et previously obtained from the same organism on January 10, 1983 (see text).

Table 6. Yields of Bmphs-d from *Chlorobium vibrioforme* (B1-20)

Bmph-d	Retention time (min)	Mass (mg)	% Mass
[Et,Et] (**1a**)	28.7	11.8	88.7
[Pr^n,Et] (**1b**)	34.1	1.1	8.3
[Bu^i,Et] (**1c**)	40.5	0.4	3.0
[Et,Me] (**1e**)	23.9	5.9	*a*

[a] [Et,Me] was the only confirmed R^5 = Me homologue present, see text.

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References

1 A. S. Holt and J. W. Purdie, *Can. J. Chem.*, 1965, **43**, 3347.
2 A. S. Holt and D. W. Hughes, *J. Am. Chem. Soc.*, 1961, **83**, 499.

3 D. W. Hughes and A. S. Holt, *Can. J. Chem.*, 1962, **40**, 171.
4 A. Jensen, O. Aasmundrud, and K. E. Eimhjellen, *Biochim. Biophys. Acta*, 1964, **88**, 466.
5 Bchls-d bearing esterifying alcohols other than farnesol have been observed (D. A. Goff and K. M. Smith, unpublished results), but have not yet been fully characterized.
6 J. L. Archibald, D. M. Walker, K. B. Shaw, A. Markovac, and S. F. MacDonald, *Can. J. Chem.*, 1966, **44**, 345.
7 T. Kemmer, H. Brockmann, Jr., and N. Risch, *Z. Naturforsch.*, 1979, **34b**, 633.
8 H. Brockmann, Jr., and R. Tacke-Karimdadian, *Liebigs Ann. Chem.*, 1979, 419.
9 N. Risch and H. Brockmann, Jr., *Liebigs Ann. Chem.*, 1976, 578.
10 A. S. Holt, D. W. Hughes, H. J. Kende, and J. W. Purdie, *Plant Cell Physiol.*, 1963, **4**, 49.
11 A. S. Holt, J. W. Purdie, and J. W. F. Wasley, *Can. J. Chem.*, 1966, **44**, 88.
12 A. S. Holt, D. W. Hughes, H. J. Kende, and J. W. Purdie, *J. Am. Chem. Soc.*, 1962, **84**, 2835.
13 K. M. Smith, L. A. Kehres, and H. D. Tabba, *J. Am. Chem. Soc.*, 1980, **102**, 7149.
14 K. M. Smith, G. W. Craig, L. A. Kehres, and N. Pfennig, *J. Chromatogr.*, 1983, **281**, 209.
15 K. M. Smith, M. J. Bushell, J. Rimmer, and J. F. Unsworth, *J. Am. Chem. Soc.*, 1980, **102**, 2437.
16 K. M. Smith, G. M. F. Bisset, and M. J. Bushell, *J. Org. Chem.*, 1980, **45**, 2218.
17 R. Tacke, Dissertation, Braunschweig, 1975.
18 K. M. Smith, in 'Porphyrins and Metalloporphyrins,' ed. K. M. Smith, Elsevier, Amsterdam, 1975, pp. 381—398.
19 J. Fajer, I. Fujita, A. Forman, L. K. Hanson, G. W. Craig, D. A. Goff, L. A. Kehres, and K. M. Smith, *J. Am. Chem. Soc.*, 1983, **105**, 3837.
20 H. Wolf, H. Brockmann, Jr., H. Biere, and H. H. Inhoffen, *Liebigs Ann. Chem.*, 1967, **704**, 208.
21 H. Scheer and J. J. Katz, in 'Porphyrins and Metalloporphyrins,' ed. K. M. Smith, Elsevier, Amsterdam, 1975, p. 417.
22 S. G. Boxer, G. L. Closs, and J. J. Katz, *J. Am. Chem. Soc.*, 1974, **96**, 7058.
23 J. J. Katz and C. E. Brown, *Bull. Magn. Reson.*, 1983, **5**, 3.
24 J. J. Katz, T. R. Janson, A. G. Kostka, R. A. Uphaus, and G. L. Closs, *J. Am. Chem. Soc.*, 1972, **94**, 2883.
25 T. J. Katz and T. R. Janson, *Ann. N.Y. Acad. Sci.*, 1973, **206**, 579.
26 K. M. Smith and J. F. Unsworth, *Tetrahedron*, 1975, **31**, 367.
27 V. Wray, U. Jürgens, and H. Brockmann, Jr., *Tetrahedron*, 1979, **35**, 2275.
28 R. J. Abraham and P. Loftus, 'Proton and Carbon-13 NMR Spectroscopy,' Heyden, London, 1979, p. 25.
29 K. M. Smith, D. A. Goff, J. Fajer, and K M. Barkigia, *J. Am. Chem. Soc.*, 1983, **105**, 1674.
30 K. M. Smith, D. A. Goff, J. Fajer, and K. M. Barkigia, *J. Am. Chem. Soc.*, 1982, **104**, 3747.
31 A. Gloe, N. Pfennig, H. Brockmann, Jr., and W. Trowitzsch, *Arch. Microbiol.*, 1975, **102**, 103.

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