Second Generation Tumour Photosensitisers: The Synthesis and Biological Activity of Octaalkyl Chlorins and Bacteriochlorins with Graded Amphiphilic Character

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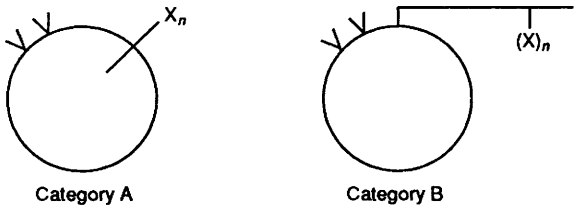
Routes to hydroxychlorin derivatives of two distinct categories (nuclear hydroxy substituent; side-chain hydroxy substituent) are developed. Such substances, covering a range of amphiphilic character depending on the number of hydroxy groups, are seen as potential sensitisers for photodynamic therapy. Osmylation of octaethylporphyrin gives the dihydroxyoctaethylchlorin **2** and the tetrahydroxybacteriochlorin **3**. Pinacol–pinacolone rearrangement of **2** gives the octaethyl-β-oxochlorin **4**, borohydride reduction of which gives the secondary alcohol **6**. This is converted *via* the bromochlorin **7** (prepared using 50% HBr/HOAc at room temperature) into a series of ethers, including those, **10**, **11**, **12**, derived from glycerol, D-glucose, and D-mannitol respectively. Alkylation of these unprotected polyols with the highly hindered bromide **7** occurs preferentially at the primary alcohol functions in each case. Some of these hydroxychlorins are found in animal assays to be highly effective sensitisers of tumour photonecrosis. In this respect the most effective compounds in each category are the most highly hydroxylated. However, the D-glucose derivative **11** proves also to be an effective sensitiser of skin and muscle, *i.e.* it does not show the selectivity shown by 5,10,15,20-tetra(*m*-hydroxyphenyl)chlorin.

Tumour phototherapy (also referred to as photoradiation therapy, PRT, and photodynamic therapy, PDT) is a form of tumour treatment in which an administered porphyrin or related system localises with some degree of selectivity in the tumour: subsequent irradiation with visible light then causes a photodynamic effect, generally regarded as being principally due to the reaction of biomolecules with singlet oxygen, and the tumour is preferentially destroyed.[1] There has been a remarkable change in direction in this field over the past 5 years or so which concerns the nature of the photosensitiser. The original work was done largely with haematoporphyrin derivative (HpD) and subsequently experimental clinical work has been almost entirely carried out with this material or one of its commercial variants (Photofrin II, Photofrin, Photosan, Photocarcinorin *etc.*). However, haematoporphyrin derivative, which is made by the action of sulfuric acid in acetic acid on haematoporphyrin,[2] is a complex variable mixture,[3] the active constituents of which have not been precisely defined, although they are thought to be oligomeric species.[4–6] A single component of high activity has not been isolated from the mixture as far as we are aware.

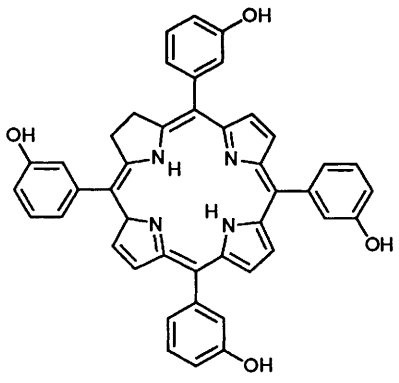
As a result there has recently been a vigorous search for 'second generation' tumour photosensitisers which are better than HpD in both tumour sensitisation and in their selectivity in causing damage to tumour in comparison with other tissue. Experience with over 100 varied porphyrin compounds submitted to the *in vivo* bioassay[4,7] led us to the view that the following properties were beneficial for our purposes: (i) the sensitiser should have a negligible or very low toxicity in the dark; (ii) it should be a single substance; (iii) the photophysical properties should be such that the quantum yield of triplet formation, φ_T, was high; the triplet energy, E_T, was greater than 94 kJ mol^{-1}; and the excitation was effectively transferred from the porphyrin triplet to triplet oxygen ($\varphi\Delta$).[8] In our experience the photophysical criteria were not difficult to meet: the next criterion, based on seemingly simpler physical chemistry, was in fact much more difficult; (iv) the solution chemistry properties (aggregation, ionic charge, solubility, partition between aqueous and lipid phases) should be such as to confer adequate selectivity without long term retention. While the mechanism of uptake is far from clear, it appeared to us that a photosensitiser with amphiphilic properties was beneficial. (v) the photosensitiser should have strong absorption in the red part of the visible spectrum, where light penetration into tissue is the greatest.[9] This criterion has had a considerable following, and modified porphyrins,[7,10] chlorins,[11,12] bacteriochlorins,[11] phthalocyanines,[13] and naphthalocyanines[14] have been prepared for assessment as tumour photosensitisers.

We embarked on the synthesis of amphiphilic chlorins of two types. Since the chlorin skeleton is essentially hydrophobic, we proposed to introduce solubilising groups, X, such as sulfonic acid or hydroxy, either at the nucleus, category A in Scheme 1, or in one or more pendant substituents, shown as category B in Scheme 1.



Scheme 1 Schematic of target molecules. The circle represents the dihydroporphyrin (chlorin) system.

One series of chlorins of category B based on the *meso*-tetraphenyl series has been described.[11] Of these, 5,10,15,20-tetra(*m*-hydroxyphenyl)chlorin **1** shows markedly improved activity and selectivity in tumour photonecrosis[15] and the first experiments in Man have now been carried out.[16] Here we describe the synthesis and assay of another series, where the chlorin oxidation level is stabilised by ββ-disubstitution. A preliminary report has appeared.[17]



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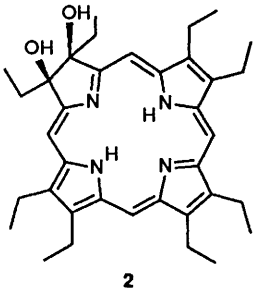

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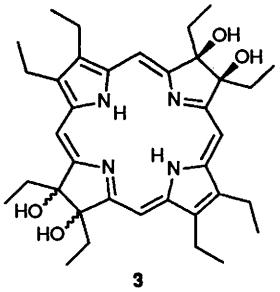
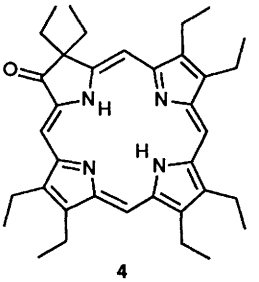


3

Synthesis.—Compounds of category A are available from the 2,3-dihydroxylation of porphyrins. Treatment of octaethylporphyrin with osmium tetraoxide (1.6 mol) gave the 2,3-dihydroxychlorin **2**[18] together with a minor amount of the 7,8,17,18-tetrahydroxybacteriochlorin **3**. As expected, these compounds showed strong sharp absorption bands in the red region [**2**, $\lambda_{max}(CHCl_3)$ 643 nm, ε 41 800; **3**, $\lambda_{max}(CHCl_3)$ 715 nm, ε 53 000]. The stereochemistry of **2** was confirmed by the X-ray structure[19] of the corresponding cyclic osmate, which was isolated as the crystalline bispyridine complex.* While each pair of diol functions in **3** is *cis*, the relative geometry of the reduced rings is not known.

The octaethyl β-oxochlorin **4** has been prepared directly from octaethylporphyrin using hydrogen peroxide in concentrated sulfuric acid.[20] The conditions in this reaction are critical, and mixtures tend to be produced which require extensive chromatographic separation. The pinacol rearrangement **2** ⟶ **4** offers a more elegant route. Various conditions have been described for this rearrangement of 2,3-dihydroxychlorins (*e.g.* $HClO_4$ in CH_2Cl_2, conc. H_2SO_4):[21] we find that concentrated sulfuric acid/fuming sulfuric acid, a reagent used for this purpose by Klotmann,[18] leads to the best results, giving a good isolated yield (75%) with little side reaction. Further osmylation of **4** furnished the dihydroxy-oxobacteriochlorin **5** in 61% yield.

Although Klotmann[18] described the reduction of the β-oxochlorin **4** to the corresponding alcohol **6** with sodium borohydride, Stolzenberg and his colleagues[22] reported that reduction did not occur with this reagent, and they used lithium triethylborohydride instead. However, we find that the reduction is smoothly effected with an excess of sodium borohydride in ethanol (4 h, 20 °C) to give **6** in an isolated yield of 87%. For present purposes **6**, **2**/**5** and **3** represent mono-, di- and tetrahydroxylated derivatives with nuclear substitution (Scheme 1, category A). Reduction of **5** would be expected to give a trihydroxy derivative: this compound was obtained, but it proved to be rather sensitive and its study was discontinued.



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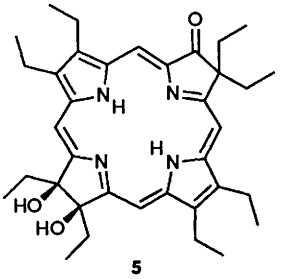
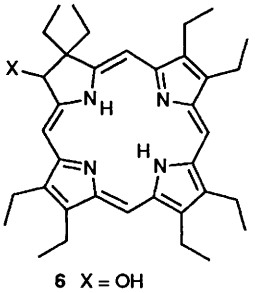
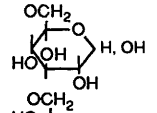
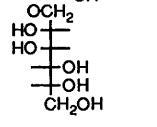


5

Compounds falling into category B (X = OH) were prepared by making ether derivatives. The alcohol **6** was treated at room temperature with 50% hydrogen bromide in acetic acid (a reagent conveniently freshly prepared by allowing acetyl bromide to react with aqueous acetic acid)[23] to give the bromide **7**. This was not isolated: the solvent was removed and the residual bromide was treated without delay with an appropriate alcohol. Thus, ethanol gave 86% of the ethoxy derivative **8** while ethylene glycol gave the 2-hydroxyethyl ether **9** in 56% yield.



6 X = OH
7 X = Br
8 X = OEt
9 X = OCH_2CH_2OH
10 X = $OCH_2CH(OH)CH_2OH$
11 X = 
12 X = 

The reactivity of the C-2 centre in the bromide **7** is already subject to steric constraints from the 3,3-dialkyl substituents, and hence polyol reagents react at their least overcrowded site. Thus, glycerol reacted at a primary, rather than the secondary, alcohol function, to give **10**. The ^1H NMR spectrum of **10** showed the expected presence of two diasteroisomers, although these were not separated. Had ether formation occurred at C-2 of the glyceryl residue, only a single pair of enantiomers would have resulted. Thus in $CDCl_3$–[$^2H_6$]-DMSO (85:15) 20-H gave two singlets (δ 9.19 and 9.18) of the same intensity; similarly, 2-H of the chlorin ring also gave two equal singlets (δ 6.27 and 6.26). The methyls of the two ethyl groups at C-3 appeared as four triplets of equal intensity representing two different methyl groups on each of two diastereoisomers.

The success with glycerol led us to extend the reaction to

* In the meantime, an X-ray structure of **2** has been published (K. M. Barkigia, C. K. Chang and J. Fajer, *J. Am. Chem. Soc.*, 1991, **113**, 7445) from which it has been concluded from the Cα–N–Cα angle of 107.7° that the nitrogen of the ring containing the *vic*-dihydroxy function does not bear a hydrogen atom. The name and structure of **2** have accordingly been modified with respect to the preliminary communication. It should be borne in mind that the preferred tautomeric forms of the other structures are not yet clearly established, but for present purposes the tautomeric representations originally used (which are generated formally by assigning lowest locants—21,23—to imino hydrogen) are retained.

produce a chlorin with a sugar-like side chain. Such compounds have occasionally been prepared in the porphyrin series,[24,25] although the compounds prepared with tumour phototherapy in mind are porphyrin glycosides.[25] This structural feature appears to us to be a potential disadvantage in application since *in vivo* glycosidase activity is likely to remove the solubilising group and cause the porphyrin to precipitate. A biologically active chlorin solubilised with a monosaccharide unit does not appear to have been reported.

The direct alkylation of monosaccharides is complicated by isomer formation, and generally protection/deprotection is required to obtain a single product in an acceptable yield.[26] However, if the alkylating agent is sufficiently sterically demanding then alkylation occurs preferentially at primary alcohol functions. Thus, tritylation of D-glucose gives the 6-*O*-trityl derivative without protection and with isolated yields of 30–50%.[27] In recent years, examples of regioselective reactions at the primary alcohol (C-6) function of D-glucose and D-glucosides have also been reported for carbamate formation;[28] and for ester and ether formation[29] using a modification of the Mitsunobu reaction. Since the alkylating agent **7** in the present example is pseudobenzylic but sterically demanding, we considered that the unprotected monosaccharide might well be preferentially attacked at the primary alcohol functions. Although reaction at the glycosidic position (as encountered with xylose, for example)[30] is a possibility, it was likely that this would be a minor pathway in the presence of excess of the sugar.

Treatment of the bromochlorin **7** with an excess of α-D-glucose in dimethylformamide, under conditions similar to those employed for the formation of the hydroxy ethers **9** and **10** already described, gave a product which was purified by chromatography on silica gel, from which it was eluted by chloroform–methanol (95:5). It formed dark green crystals (64% yield) from chloroform–hexane: elemental analysis and accurate measurement of the molecular ion indicated a molecular formula of $C_{42}H_{58}N_4O_6$. The ether is formulated as the 6-oxy derivative **11** on the basis of the earlier arguments. The ^1H NMR spectrum in $CDCl_3$–[$^2H_6$]-DMSO (85:15) revealed the expected mixture of diastereoisomers. Thus, in the lowfield *meso*-proton region the signal assigned to 20-H, which is most closely placed to the chiral glucose substituent, appeared as four signals: δ 9.21 and 9.23 (0.2 H each) and 9.26 and 9.28 (0.3 H each). This indicates four diastereoisomeric systems, the two centres of variance being C-2 of the chlorin and the anomeric carbon of the sugar. Since only four isomers are detected, we formulate the product in the pyranose form, the alternative furanose, if present, not being detected in the NMR spectrum under these conditions. The signal for 10-H and 15-H appeared as a broad singlet (δ 9.75), but the other *meso*-hydrogen (C-5) flanking the reduced ring gave two closely spaced signals (δ 8.77, 8.76) again in the ratio of *ca.* 3:2 respectively. Thus, the four diastereoisomers occur in two sets in the ratio of 3:2. Attempts to observe the mutarotation of **11** in methanol were made: the results were positive but being at the limits of measurement of the circular dichroism spectrometer available (low rotation, high extinction) are not regarded as convincing. However, evidence for anomerisation in methanol–water (10:1) was observed on reversed phase HPLC. Immediately after being made up, the solution of diastereoisomeric chlorin sugars showed two peaks. The relative abundance of the most polar component (*i.e.* the first peak) changed from 38 to 50% on storage of the solution in the dark at room temperature for 48 h.

In a similar way, D-mannitol gave the corresponding primary ether **12**. In this example, there is no glycosidic position, and only two diastereoisomers were detected. These were seen in the ^1H NMR spectrum, where the signals corresponding to the C-20 protons appeared at δ 9.05 and 9.07, the respective ratio of the diastereoisomers being *ca.* 0.55:0.45.

Thus, in compounds **9–12** we had available chlorins of category B with a polyol side chain. These compounds were not freely soluble in water, but they were non-ionic amphiphilic molecules of the type being sought.

Biological Assay.—The hydroxylated compounds were subjected to an *in vivo* bioassay for tumour photonecrosis. This has been described in detail elsewhere.[4,7] Essentially, an animal bearing a standardised tumour (PC6 myeloma) on its flank is treated with a known dose of the photosensitiser: after 24 h the tumour is irradiated with a standard light dose (10 J cm^{-2}) delivered at the maximum of the lowest energy absorption band. After a further 24 h the animal is injected with a dyestuff (Evans Blue) to delineate the vascular system. It is then sacrificed, the tumour is fixed and sectioned at right angles to the flank, and the depth of photonecrosis—tissue into which the blue dye has not penetrated—is measured under a low power microscope. This gives a numerical index of the activity of the sensitiser in causing photodestruction of tumour tissue (*i.e.* in tumour photonecrosis).

Table 1 shows the biological assay results for several compounds of categories A and B. The β-oxochlorin **4** was inactive, and the monohydroxychlorin **6** gave erratic results. The latter were considered to be due to problems associated with low solubility in aqueous systems, and for this reason the monohydroxy compound in category B (the glycol ether **9**) was not subjected to bioassay. The trihydroxy compound produced by reduction of the dihydroxy β-oxochlorin **5** with sodium borohydride had originally been tested biologically,[17] but further chemical study showed that solutions of this material were particularly labile, predicating a poor shelf-life. Work on it was, therefore, discontinued.

However, the other compounds did not show this sensitivity, and those chlorins containing two or more hydroxy functions in the nucleus or in a side chain proved to be highly effective sensitisers of tumour photonecrosis. The most highly hydroxylated compounds, the tetrahydroxybacteriochlorin **3** and the mannityl chlorin ether **12** proved to be the most effective: indeed the dose of the latter had to be reduced to 0.75 μmol kg^{-1} (*ca.* 0.01 mg per animal) in order to bring it on to our operating scale. These substances are all much more effective in promoting tumour photonecrosis in our system than is Photofrin II, data on which are included for comparison.

However, for a clinically useful photosensitiser, activity in the photonecrosis of tumour tissue is only the first step. What is needed is *selectivity* of photodamage. We have examined this question for one of the photosensitisers described here (the chlorinylglucose **11**) and the results are not promising. Compound **11** was administered intravenously in ethanol–polyethylene glycol–water and a comparison of photodamage to tumour implants and to normal skin and muscle was made. Damage to the normal tissues was based on the development of oedema, with increase in tissue weight.[7] The results for this series of comparisons are shown in Table 2. The conclusions from Table 1 (which refers to intraperitoneal administration) are confirmed: the chlorinylglucose **11** is an effective tumour photosensitiser. But it is also very effective in photosensitising skin and muscle. This lack of selectivity means that this substance cannot be regarded as a promising photosensitiser for photodynamic therapy: it causes general sensitisation. It emerges, however, that other chlorins of category B (e.g. **1**) are much more selective.[15]

Experimental

General.—Electronic spectra were measured on a Perkin-Elmer 552 spectrometer; ^1H NMR spectra were measured in $CDCl_3$, unless otherwise stated, with a Bruker AM250. *J* Values

Table 1 Tumour photonecrosis with some hydroxyhydroporphyrins[a]

Structure	Functionality	Dose (μmol kg^{-1})	λ[b] nm	Depth of photonecrosis[c] [mm ± SE (n)]
Category A				
6	β-Hydroxy	12.5	646	Results erratic
2	β,β′-Dihydroxy	12.5	645	5.69 ± 0.92 (9)
		1.56	645	0.86 ± 0.33 (9)
5	β,β′-Dihydroxy-β″-oxo	12.5	696	2.63 ± 0.60 (6)
		8.12	696	0.95 ± 0.59 (5)
3	β,β′,β″,β‴-Tetrahydroxy	25.00	712.5	>7.29 ± 0.42 (6)
		3.12	712.5	2.72 ± 0.82 (6)
Category B				
10	Chain dihydroxy	12.5	646	4.36 ± 0.48 (7)
11	Chain tetrahydroxy	12.5	647	6.43 ± 0.53 (7)
12	Chain pentahydroxy	12.5	645	*d*
		3.125	645	>6.38 ± 1.02((6)[e]
		0.75	645	3.58 ± 0.75 (6)
Photofrin II		100	625	3.03 ± 0.45 (8)
		50	625	1.94 ± 0.30 (12)

[a] The bioassay is described in refs. 4 and 7. The photosensitiser was introduced intraperitoneally in dimethyl sulfoxide. [b] Wavelength of irradiation from tunable dye laser (Oxford Lasers Cu 10 copper vapour laser pumping a DL 10K dye laser), light dose 10 J cm^{-2}. [c] Index of biological activity of the photosensitisers against tumours *in vivo*: the number records the depth (in mm) of destruction of a sub-cutaneous tumour of a selected standard size following administration of the drug at the stated dose and irradiation with monochromatic light under standardised conditions. The index is quoted with a standard error (SE) which refers to the number of tumours in brackets.[4] [d] All animals died. [e] Two animals died.

Table 2 Sensitisation of various tissues using the chlorinylglucose **11**[a]

Tissue: Parameter measured	Tumour: Depth of necrosis (mm)	Skin:[b] Increase in weight due to oedema(%)	Muscle:[b] Increase in weight due to oedema(%)
Dose of photosensitiser (μmol kg^{-1})			
0	—	0 ± 4.8	—
0.20	0(8)[c]	5.24 ± 9.9	−2.9 ± 2.3
0.39	0(8)	—	19.6 ± 2.9
0.78	0.25 ± 0.14 (4)	22.5 ± 8.9	62.0 ± 3.7
1.56	1.69 ± 0.49 (8)	—	57.0 ± 6.1
3.12	3.88 ± 1.58 (6)	68.6 ± 15.2	—

[a] The bioassay is described in ref. 7. The photosensitiser was introduced intravenously in ethanol–polyethylene glycol–water. Light dose 10 J cm^{-2} at 647 nm. [b] Average of five experiments. [c] Number of experiments in brackets.

are given in Hz. Mass spectra were recorded on a Kratos MS50 or a VG ZAB-SE: relative abundances and assignments are given in parentheses. Unless otherwise stated ionisation was by electron impact: for fast atom bombardment (FAB) measurements the matrix was *p*-nitrobenzyl alcohol. M.p.s were measured on a hot-stage apparatus, and are uncorrected. Reactions were monitored using TLC on Merck Kieselgel 60 silica gel plastic sheets. Column chromatography was carried out on Merck Kieselgel 60 silica gel (0.040–0.063 mm). A Spectra Physics 8750 instrument equipped with a CE 272 variable wavelength detector was employed for high performance liquid chromatography (HPLC) using a Technopak 10 C18 column (300 × 3.9 mm diam.) eluted with methanol–water (10:1).

50% HBr in acetic acid (w/v) was made as follows. Water (1.10 cm^3, degassed) was added dropwise to a mixture of acetyl bromide (7.5 g, 4.51 cm^3) and acetic acid (1.23 cm^3) under nitrogen at 0 to −10 °C (CO_2–$CHCl_3$) with stirring. After 10 min the reagent was ready for use.

Osmylation of Octaethylporphyrin.—Octaethylporphyrin (700 mg, 1.31 mmol) in dichloromethane (120 cm^3) and pyridine (0.5 cm^3) was stirred with osmium tetraoxide (545 mg, 2.14 mmol) under nitrogen in the dark for 90 h. The solvent was removed under reduced pressure and the residue was dissolved in methanol (100 cm^3) and dichloromethane (30 cm^3) and hydrogen sulfide was passed through the solution for 30 min. The precipitate was filtered off (glass sinter) and the pad was washed with chloroform, and the total filtrate was taken to dryness. The residue was dissolved in chloroform (5 cm^3) and methanol (50 cm^3) was added to precipitate some of the unchanged octaethylporphyrin. The solution was filtered and the pad was washed with methanol (100 cm^3) and the total filtrate was taken to dryness. The residue was chromatographed on silica (300 × 25 mm diam.) with chloroform–acetone (98:2). After the elution of unchanged starting material (total recovery *ca.* 20%), the major product, 2,3,7,8,12,13,17,18-octaethyl-2,3-dihydroxy-22*H*,24*H*-chlorin **2** (420 mg, 56%), was obtained as dark blue prisms, m.p. 213 °C (decomp.) from dichloromethane–hexane [lit.,[18,31] 219–220 °C from ether; 218 °C (decomp.) from methanol–chloroform]; $\lambda_{max}(CHCl_3)$/nm 393 (ε 170 000), 497 (12 600), 522 (2 500), 589 (3 800), 613 (3 500) and 643 (41 800).

The next significant band to emerge was 2,3,7,8,12,13,17,18-*octaethyl*-7,8,17,18-*tetrahydroxybacteriochlorin* **3** (17 mg, 2%) which crystallised from hexane as dark green prisms, m.p. 170–172 °C (decomp.) [Found: C, 70.2; H, 8.1; N, 8.9%; M + 1$^+$ (FAB), 603. $C_{36}H_{50}N_4O_4.H_2O$ requires C, 69.95: H, 8.45; N, 9.05%; $C_{36}H_{50}N_4O_4$ requires *M*, 602]; a satisfactory electron impact spectrum could not be obtained; $\lambda_{max}(CHCl_3)$/nm 355 (ε 82 600), 376 (113 000), 442 (2600), 471 (6200), 501 (18 900), 599 (1100), 644 (3800), 680 (6600) and 715 (53 000); δ 8.91 (s, 4H, *meso*-H), 3.83 (q, *J* 7.5, 8 H, 2,3,12,13-CH_2CH$_3$), 3.30 (s, 4 H, OH), 2.57 (m, 8 H, 7,8,17,18-CH_2CH$_3$), 1.75 (t, *J* 7.5, 8 H, 2,3,12,13,-CH$_2$CH_3), 1.10 (t, *J* 7.5, 12 H, 7,8,17,18-CH$_2$CH_3) and −2.18 (s. 2 H, 2 NH); *m/z* (FAB) 604 (46, M + 2), 603 (100, M + 1), 602 (16), 587 (5), 586 (11), 556 (7) and 555 (5).

Pinacol Rearrangement of the 2,3-*Dihydroxychlorin* **2**.—(*a*)[21] The 2,3-dihydroxychlorin (100 mg, 0.18 mmol) in dichloromethane (60 cm^3) was stirred with 70% perchloric acid (1 cm^3) for 30 min at 20 °C. The mixture was washed in turn with water, aqueous sodium hydrogen carbonate and water. The organic layer was separated, dried (Na_2SO_4), and evaporated. The residue was chromatographed on silica gel (250 × 35 mm diam.) with dichloromethane. The faster moving band was

removed and the major band was worked up to give 3,3,7,8,12,13,17,18-octaethyl-2-oxochlorin **4** (71 mg, 72%) as rosettes of needles, m.p. 247–250 °C (lit.,[20] m.p. 246–248 °C).

(*b*) Concentrated sulfuric acid (2.5 cm^3) and fuming sulfuric acid (20% SO_3; 2 cm^3) were added to the 2,3-dihydroxychlorin (200 mg) and the resulting green solution was kept at room temperature for 7 min, poured into a large excess of ice and extracted with chloroform. The organic layer was washed with water, dried (Na_2SO_4), taken to dryness, and the residue was crystallised from chloroform–hexane. The product **4** (73 mg, 75%) was identical with an authentic sample.[20]

2,3,8,8,12,13,17,18-*Octaethyl*-17,18-*dihydroxy*-7-*oxobacteriochlorin* **5**.[32]—The 2-oxochlorin **4** (100 mg, 0.18 mmol) in dichloromethane (40 cm^3) and pyridine (1.6 cm^3) was stirred at room temperature under nitrogen with osmium tetraoxide (100 mg, 0.39 mmol) for 72 h. The solution was evaporated and the residue was dissolved in methanol (40 cm^3) and dichloromethane (12 cm^3). Hydrogen sulfide was bubbled through the resulting solution (15 min) and the precipitate was filtered off and the filtrate was taken to dryness. The residue was chromatographed on silica gel (150 × 15 mm diam.) with chloroform to give 2,3,8,8,12,13,17,18-octaethyl-17,18-dihydroxy-7-oxobacteriochlorin (65 mg, 61%) as dark green crystals, m.p. 134–136 °C (d) from hexane (Found: M^+, 584.373. Calc. for $C_{36}H_{48}N_4O_3$ *M*, 584.373); λ_{max} ($CHCl_3$)/nm 396 (ε 81 800), 415 (98 000), 473 (3800), 497 (8500), 536 (2700), 581 (1700), 632 (5500), 659 (6800) and 693 (50 900); δ 9.56 (s, 1 H, *meso*-H), 8.92 (s, 2 H, *meso*-H), 8.77 (s, 1 H, *meso*-H), 3.84 (m, 8 H, CH_2CH_3 at 2,3,12,13), 3.66, 3.00 (s, s, 1 H each, 2 × OH), 2.45–2.70 (m, 8 H, CH_2CH_3 at 8,17,18), 1.77 (m, 12 H, CH_2CH_3 at 2,3,12,13), 0.95, 0.88, (t, t, *J ca.* 7, each CH_2CH_3 at 17,18), 0.42, 0.38 (t, t, *J ca.* 7, each 3 H, CH_2CH_3 at 8α,8β) and −1.90, −1.96 (s, s, 2 × NH); *m/z* 584 (1, M), 568 (8), 567 (40), 566 (M-H_2O, 100), 550 (8), 549 (24), 548 (58), 538 (12), 537 (29), 526 (6), 525 (15), 523 (3), 522 (7), 519 (11) and 504 (5).

3,3,7,8,12,13,17,18-*Octaethyl*-2-*hydroxychlorin* **6**.—Octaethyloxochlorin (61 mg, 0.11 mmol) dissolved in dichloromethane (5 cm^3) and ethanol (50 cm^3) was stirred with sodium borohydride (1.0 g) for 4 h at room temperature. The mixture was neutralised with 5% HCl, poured into water and extracted with chloroform. The chloroform solution was washed with water, dried (Na_2SO_4) and evaporated. The residue was chromatographed on a silica gel column (150 × 15 mm diam.) with chloroform to give 2-hydroxy-3,3,7,8,12,13,17,18-octaethylchlorin (53 mg, 87%) as purple rosettes, m.p. 217–219 °C (lit.,[18] 205–206 °C; lit.,[22] not given) (Found: C, 78.2; H, 8.7; N, 10.7%; M^+, 552.383. Calc. for $C_{36}H_{48}N_4O$: C, 78.2; H, 8.75; N, 10.15%; *M*, 552.383); λ_{max}($CHCl_3$)/nm 391 (ε 188 000), 492 (13 200), 520 (3200), 589 (4500), 613 (4070) and 642 (43 700); ν_{max}(KBr)/cm^{-1} 3445, 3340 and 1615; *m/z* 553 (18), 552 (44, M), 551 (8), 550 (17), 537 (11), 536 (30), 535 (42), 534 (100), 523 (11), 520 (6), 519 (15) and 507 (13).

2-*Ethoxy*-3,3,7,8,12,13,17,18-*octaethylchlorin* **8**.—The foregoing hydroxychlorin **6** (10 mg) was dissolved in 50% HBr in HOAc (2 cm^3) under nitrogen and the solution kept at room temperature for 1 h. The solvent was removed under reduced pressure (vacuum pump): the resulting crude 2-bromo-3,3,7,8,12,13,17,18-octaethylchlorin **7**, a dark green solid, could be stored at 4 °C. The product was dissolved in ethanol (5 cm^3): after 5 min the solvent was removed under reduced pressure, and the residue was chromatographed on silica gel (100 × 15 mm diam.) with dichloromethane to give 2-*ethoxy*-3,3,7,8,-12,13,17,18-*octaethylchlorin* as a dark green solid (9 mg, 86%), m.p. 197–198 °C (from hexane) (Found: M^+, 580.414. $C_{38}H_{52}N_4O$ requires *M*, 580.414); λ_{max}($CHCl_3$)/nm (relative absorbances) 389 (3.11), 494 (0.33), 523 (0.12), 543 (0.19), 589 (0.12), 614 (0.14) and 643 (1.0); δ 9.73 (s. 2 H, 10-, 15-H), 9.12 (s, 1 H, 20-H), 8.73 (s. 1 H, 5-H), 6.26 (s, 1 H, 2-H), 4.27 (m, 2 H, OCH_2CH_3), 3.88–4.02 (m, 12 H, 6 × CH_2CH_3), 2.27–2.65 (m, 4 H, 3,3-CH_2CH_3), 1.79 (m, 18 H, 6 × CH_2CH_3), 1.58 (t, 3 H, OCH_2CH_3), 0.92, 0.63 (t, t, 3 H each, 3,3-CH_2CH_3) and −2.55 (s. 2 H, 2 × NH); *m/z* 582 (5), 581 (44), 580 (100, M), 552 (5), 551 (14), 550 (7), 536 (12), 535 (17), 534 (13), 523 (4), 522 (8), 508 (6), 507 (17), 506 (6), 493 (4) and 491 (6).

3,3,7,8,12,13,17,18-*Octaethyl*-2-(2-*hydroxyethoxy*)*chlorin* **9**.—The bromooctaethylchlorin **7** obtained from the 2-hydroxychlorin **6** (5 mg) as before was dissolved in ethylene glycol (0.5 cm^3) and kept for 30 min at room temperature. The mixture was poured into water (100 cm^3) and extracted with chloroform (2 × 50 cm^3). The chloroform extract was washed with water (4 × 100 cm^3), dried (Na_2SO_4), and taken to dryness. The residue was crystallised from dichloromethane–hexane to yield 3,3,7,8,12,13,17,18-*octaethyl*-2-(2-*hydroxyethoxy*)*chlorin* **9** (3 mg, 56%) as a dark green solid, m.p. 216–218 °C (Found: C, 76.2; H, 8.8; N, 9.3%. $C_{38}H_{52}N_4O_2$ requires C, 76.45; H, 8.8; N, 9.4%); λ_{max}($CHCl_3$)/nm 391 (ε 201 000), 494 (13 100), 521 (2100), 542 (450), 588 (3000), 612 (2700) and 641 (47 500); δ 10.07 (s. 2 H, 10-, 15-H), 9.46 (s, 1 H, 20-H), 9.04 (s, 1 H, 5-H), 6.40 (s, 1 H, 2-H), 4.37 (m, 2 H, OCH_2CH_2OH), 4.20 (m, 2 H, OCH_2CH_2OH), 3.99–4.06 (m, 12 H, 6 × CH_2CH_3), 2.39–2.68 (m, 4 H, 3,3-CH_2CH_3), 1.81 (m, 18 H, 6 × CH_2CH_3), 0.98, 0.74 (t, t, *J* 7, 3,3-CH_2CH_3) and −2.90, −3.03 (s, s, 1 H each, 2 × NH); *m/z* (FAB) 599 (9), 598 (38), 597 (100, M + 1), 596 (81), 595 (24), 581 (5), 551 (5), 535 (10), 521 (4), 507 (21) and 491 (6).

2-(2,3-*Dihydroxypropoxy*)-3,3,7,8,12,13,17,18-*octaethylchlorin* **10**.—The bromochlorin **7** from the hydroxychlorin **6** (12 mg, 0.022 mmol) was kept in a mixture of dimethylformamide (1 cm^3) and glycerol (1 cm^3) at room temperature for 1 h. The solution was poured into water (200 cm^3) and extracted with chloroform (200 cm^3). The organic layer was washed with water (5 × 200 cm^3), dried (Na_2SO_4), taken to dryness and the residue chromatographed on silica gel (200 × 15 mm diam.) with chloroform followed by chloroform–methanol (99:1). The major product was eluted and crystallised from hexane to give the *title compound* **10** (11.3 mg, 83%, mixed diastereoisomers) as a dark green solid, m.p. 205–207 °C (Found: M + H^+, 627.427. $C_{39}H_{54}N_4O_3$ + H requires *M*, 627.427); λ_{max}($CHCl_3$)/nm 391 (ε 194 000), 494 (13 600), 521 (2300), 587 (3100), 613 (3000) and 642 (49 000); δ ($CDCl_3$–[$^2H_6$]-DMSO 85:15) 9.74 (s, 2 H, 10-, 15-H), 9.19, 9.18 (s, s, 0.5 H each, 20-H of two diastereoisomers), 8.76 (s, 1 H, 5-H), 6.27, 6.26 (s, s, 0.5 H each, 2-H of two diastereoisomers), 4.30 (m, 2 H, CH_2OR), 4.15 (m, 1 H, CHOH), 3.8–4.0 (m, 14 H, 6 × CH_2CH_3 + CH_2OH), 3.64 (m, OH), 2.62 (br s, OH), 2.3–2.7 (m, 4 H, 3,3-CH_2CH_3), *ca.* 1.8 (m, 18 H, 6 × CH_2CH_3), 0.90, 0.88, 0.68, 0.66 (4 × t, 1.5 H each, 3,3-CH_2CH_3) and −2.67 (s, 2 H, 2 × NH); *m/z* (FAB) 627 (100, M + 1), 535 (28) and 507 (25).

6-O-(3,3,7,8,12,13,17,18-*Octaethylchlorin*-2-*yl*)-D-*glucopyranose* **11**.—The bromochlorin **7** obtained as before from the hydroxychlorin **6** (18 mg, 0.033 mmol) was stirred with an excess of α-D-glucose (250 mg) in dimethylformamide (1 cm^3) for 1 h at room temperature. The solvent was removed under reduced pressure, aqueous sodium sulfate was added, and the mixture was extracted with chloroform. The organic layer was dried (Na_2SO_4), taken to dryness, and the residue chromatographed on silica gel (200 × 15 mm diam.), eluting with chloroform and then chloroform–methanol (95:5). Some minor fast-moving bands were followed by the major component, which was crystallised from chloroform-hexane to give the *title compound* **11** as a mixture of diastereoisomers (15 mg, 65%), a

dark green solid, m.p. 189–190 °C (Found: C, 69.9; H, 8.1; N, 8.0%; M^+, 714.436. $C_{42}H_{58}N_4O_6$ requires C, 70.55; H, 8.2; N, 7.85%; *M*, 714.436); $\lambda_{max}(CHCl_3)$/nm 391 (ε 212 000), 493 (13 900), 521 (2300), 546 (410), 589 (3300), 612 (2700) and 641 (48 700); δ($CDCl_3$–[$^2H_6$]-DMSO 85:15) 9.75 (s, 2 H, 10-, 15-H), 9.28, 9.26, 9.23, 9.21 (4 × s, total 1 H, ratio 3:3:2:2, 20-H of four diastereoisomers), 8.77, 8.76 (s, s, total 1 H, ratio *ca.* 3:2, 5-H of diastereoisomers), 6.34, 6.26, 6.21 (3 × s, total 1 H, ratio 3:5:2, 2-H), *ca.* 6.1 (br m, exchangeable, OH), 5.75 (br s, exchangeable, OH), 5.69 (br s, exchangeable, OH), 5.34, 5.27, (2 × br s, 0.6 H, 1-H of α-anomer), 4.62 (br m, 0.4 H, 1-H of β-anomer), 4.50 (m, 2 H, CH_2O-C-2), 4.3–3.1 (br m, tertiary H of glucose ring at C-2, C-3, C-4, C-5), 3.8–4.1 (complex m of overlapping q, 6 × ArCH_2CH_3), 2.3–2.7 (complex m of overlapping q, 3,3-CH_2CH_3), 1.7–1.9 (complex m of overlapping t, 6 × ArCH_2CH_3), 0.6–0.9 (complex m of overlapping t, 3,3-CH_2CH_3) and −2.68 (2 H, 2 × NH). The assignments of the anomeric hydrogen signals are based on the coupling constants with the glucose 2-H measured after shaking the sample with D_2O (α-anomer 3.75 Hz, β-anomer 7.5 Hz);[33] *m/z* (FAB) 715 (82, M + 1), 714 (100, M), 551 (12), 535 (45, M-$C_6H_{11}O_6$), 507 (46), 491 (17), 477 (14) and 461 (12).

3,3,7,8,12,13,17,18-*Octaethyl*-2-(D-*mannit*-1-*yloxy*)*chlorin* **12**.—The bromochlorin **7** obtained as before from the hydroxychlorin **6** (39 mg, 0.071 mmol) was stirred with an excess of D-mannitol (300 mg) in dry dimethylformamide (20 cm^3) at room temperature, under nitrogen for 2 h. After this aqueous sodium sulfate was added, and the mixture extracted with chloroform. The organic layer was washed with water, dried, taken to dryness and the residue chromatographed on silica gel (350 × 30 mm), eluting with chloroform and chloroform–methanol (10:1). The major product was eluted and crystallised from hexane to give the title compound as a mixture of two diastereoisomers (9 mg, 18%). Dark green solid, m.p. 143–145 °C (Found: C, 66.45; H, 8.4, N, 7.35%; M + 1^+ = 717.459. $C_{42}H_{60}N_4O_6$ requires C, 70.4; H, 8.4; N, 7.84%; M + 1 = 717.459. $C_{42}H_{60}N_4O_6.2H_2O$ requires C, 67.0; H, 8.55; N, 7.45%); $\lambda_{max}(CHCl_3)$/nm 392 (ε 182 000), 495 (13 100), 522 (5 000), 589 (3700) and 641 (44 900); ν_{max}(KBr)/cm^{-1} 3600–3000, 3340 and 1616; δ 9.71 (br s, 2 H, 10-, 15-H), 9.05, 9.07 (2 × s, ratio 0.55:0.45 total 1 H, 20-H of two diastereoisomers), 8.66 (br s, 1 H, 5-H), 6.00 (br s, 1 H, 2-H), 2.6–4.3 (complex m, overlapping CH and OH of mannityl chain), 3.6–4.0 (m, 6 × ArCH_2CH_3), 2.1–2.6 (m, 3,3-CH_2CH_3), 1.7–1.9 (m, 6 × Ar CH_2CH_3), 0.55–0.74 (m, 3,3-CH_2CH_3) and −2.70 (br s, 2 H, 2 × NH); *m/z* (FAB) 717 (82, M + 1), 716 (100, M) 551 (11), 535 (65, M − mannityloxy), 523 (10), 507 (53), 491 (17), 477 (10) and 461 (6).

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