Effect of Substituents in OsO4 Reactions of Metallochlorins Regioselective Synthesis of Isobacteriochlorins and Bacteriochlorins

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Abstract: *A first regioselective synthesis of bacteriochlorin by hydroxylation of metallated chlorin with OsO4 is discussed. This is the first example for the preparation of metallobacteriochlorin. This methodology can also be used for the preparation of unsymmetrical isobacteriochlorins by introducing electron-withdawing substituent(s) at the adjacent position(s) of the reduced ring. The Zn(II) vic-dihydroxybacteriochlorin obtained from 2-formyl-purpurin methyl ester showed long wavelength absorption at 828 nm which seems to be associated with electronic interactions of the substituents with the π-system of the macrocycle.* Copyright © 1996 Elsevier Science Ltd

Isobacteriochlorins and bacteriochlorins have been known for some time,[1] but their biological significance had not been recognized until recently. The dihydroxyporphyrin structure originally proposed by Barrett [2] for heme *d* isolated from *Aerobacter aerogenes* was later reinvestigated by Timkovich et al.[3] to show that it is in fact a derivative of 5,6-dihydroxyprotochlorin IX. It has also been reported by Sotiriou and Chang[4] that heme *d₁* obtained from *Pseudomonas aeruginosa* and *Paracocis denitrificans* is a dioxo-isobacteriochlorin. In recent years, it has also been shown that some of the oxochlorins, dioxobacteriochlorins and their *vic-* dihydroxy precursors can also be used as photosensitizers for the treatment of cancer by photodynamic therapy (PDT).[5,6] In preliminary studies, certain naturally occurring bacteriochlorins were found to show *in vivo* photosensitizing activity.[7] However, due to their unstable nature (readily converted to chlorins), further studies were hampered. Efforts are currently being made by various investigators to prepare stable bacteriochlorins as effective photosensitizers for (PDT) and as models for photosynthetic reaction centers.

Chang and coworkers[8] were the first to show that free base and metallated octaethylchlorin (without any electronwithdrawing substituents), on reacting with OsO4, can be converted into the corresponding *vic-* dihydroxy bacteriochlorins and isobacteriochlorins. This approach was later extended by us[9] and others,[10] and similar results were obtained.

It is now well accepted that the activity of the pyrrole subunit in the chlorin systems is remarkably influenced by the presence of center metal in directing the formation of β,β-dihydroxyisobacteriochlorins. In our previous report, we have shown that the regiospecificity of the pyrrole subunit in OsO4 oxidation is also affected by the presence of electron-withdrawing groups. For example, in free base porphyrins, presence of an acetyl group at the 2- or 4- position of the macrocycle deactivates that particular pyrrole unit(s) for OsO4 oxidation.[9]

In our attempts to investigate the effect of electron-withdrawing groups in regiospecificity of OsO4 oxidation of metallated chlorins, Ni(II) mesochlorin e6 trimethyl ester **1**, Ni(II) mesopurpurin-18 methyl ester **4**, Zn(II) 3-formyl purpurin-18 methyl ester **8** contain electron withdrawing substituent(s) at the adjacent pyrrole subunit(s) of the reduced ring were used as substrates. The starting materials were prepared by following known methodology.[11]

Reaction of Ni(II) chlorin e_6 1 with OsO_4 in CH_2Cl_2 in the presence of pyridine gave the osmate ester which was reduced to *vic-* dihydroxy isobacteriochlorin with gaseous H_2S. The mixture was separated into two isomers **2** [2,3-*vic-* dihydroxy groups (ring A)] and **3** [12, 13-*vic-* dihydroxy (ring C)] by preparative plates.
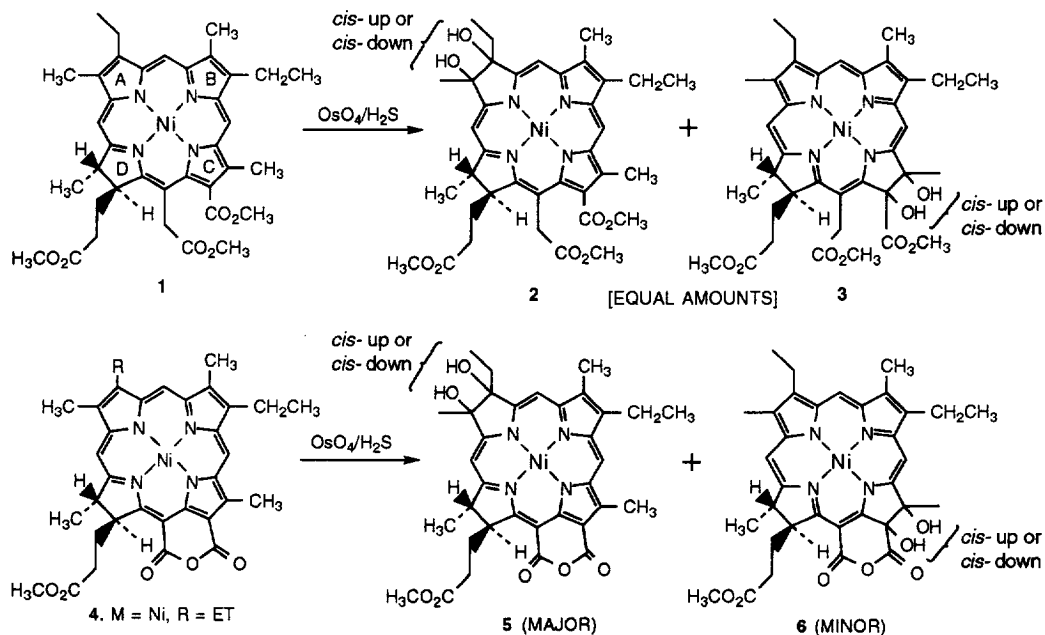
In each isomer the dihydroxy groups are arranged in a planar platform with *cis-* diols up- or down relative to ring D (*trans-* reduced). To our surprise, the electron-withdrawing nature of methoxycarbonyl substituent attached at position-13 of the macrocycle did not produce any significant influence in deactivating that particular pyrrole unit, and *vic-* dihydroxy isobacteriochlorins **2** and **3** were obtained in almost equal amounts. On the other hand, when Ni(II) mesopurpurin-18 methyl ester **4** (containing a fused anhydride ring as electron withdrawing substituent) was used as a substrate, *vic-* dihydroxy isobacteriochlorins **5** (ring A diol) and **6** (ring C diol) were isolated in 4:1 ratio. Thus, compared to the -CO_2CH_3 group, the fused anhydride ring was more efficient in deactivating the particular pyrrole subunit and showed a significant effect in directing the outcome of the hydroxylation reaction. Under similar reaction conditions, 2-formylpurpurin-18 methyl ester **7** as well as its Zn(II) complex **8** gave mainly related *vic-* dihydroxybacteriochlorins **9** and **10**[12] as diastereomeric mixtures with long wavelength absorption at 828 nm and 813 nm respectively in > 60% yield. Thus, these results suggest that on introducing electron withdrawing groups at the adjacent ring(s) of the reduced ring, regiospecific synthesis of metallated bacteriochlorins can be achieved. Several attempts were made to convert the *vic-* dihydroxybacteriopurpurin **10** into the corresponding Zn (II) complex **9**, but without much success, which might be due to severe distortion in the planarity of the macrocycle **10**. Leaving the reaction mixture with $Zn(OAc)_2$/MeOH in refluxing THF for 5 days gave the corresponding metallated analog in only 10% yield.

The formation of these novel bacteriochlorins and isobacteriochlorins were clearly confirmed by their well

known characteristic UV-VIS properties. Compared to Zn(II) 2-formylpurpurin-18 methyl ester **8** (711 nm), bacteriochlorin **9** gave a remarkable hypsochromic shift with long wavelength absorption at 828 nm. Hence, the observed hypsochromic shifts seem to be associated with electronic interactions of the substituents with
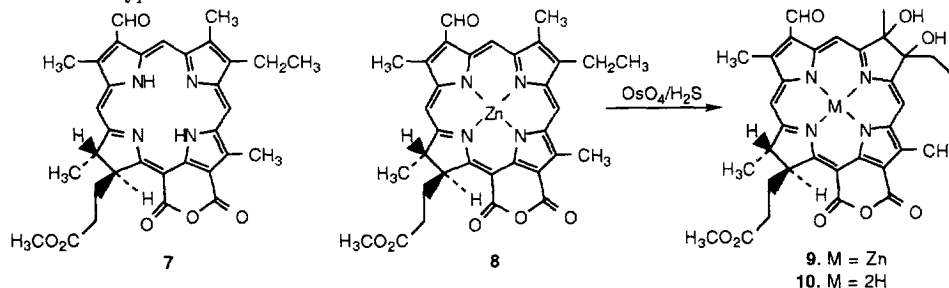
the π system of the pigments. Interestingly, compared to mesopurpurin **4** (666 nm), isobacteriochlorin **5** (584 nm) and **6** (587 nm) produced bathochromic shifts of 82 and 79 nm respectively (see Fig. 1).
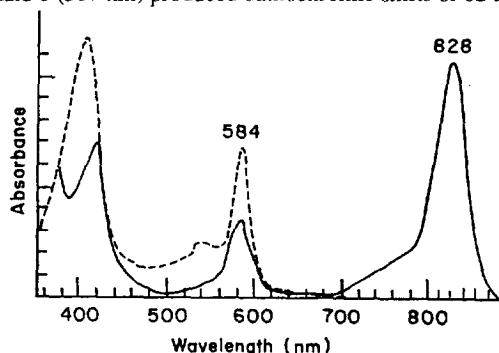

Fig. 1: Optical spectra (in CH$_2$Cl$_2$) of Ni (II) isobacterio-chlorin **5** (------) and Zn (II) bacteriochlorin **9** (———).

The structures of all the newly synthesized compounds were also confirmed by NMR and mass spectroscopy. For example, compared to 12,13-*vic*-dihydroxy isobacteriochlorin **3**, the resonances of the -C$_2$H$_5$ group attached at position-3 in 2,3-*vic*-dihydroxy isobacteriochlorin **2** showed upfield shift in the NMR spectra. The 2-CH$_3$ resonances in **2** were also upfield and were observed at δ 1.80 ppm. Similar shifts were observed for 12-CH$_3$ (δ 1.40 ppm) and 13-CO$_2$CH$_3$ (δ 3.23 ppm) protons in 12,13-*vic*- dihydroxybacteriochlorin **3**.[13]

By following a similar approach, the structure of ring A and ring C diols- **5** and **6** obtained from Ni(II) mesopurpurin-18 methyl ester **4** were also confirmed.[12] The novel isobacteriochlorins and bacteriochlorins were isolated as diasteriomeric mixtures.[13] However, unlike isobacteriochlorins, in the NMR spectrum of bacterio-purpurin **9**, all the resonances were observed in two sets. The structures of newly synthesized compounds were also confirmed by mass spectrometric analysis.

In summary, we have shown that a regiospecific synthesis of bacteriochlorins can be achieved by hydroxylation of not only chlorins but also their metallated analogs by introducing a strong electron withdrawing substituent(s) at the adjacent pyrrole units of the reduced ring. Introduction of such electron- withdrawing substituents at appropriate position(s) could also help to achieve a regioselective synthesis of unsymmetrical isobacteriochlorins, which are otherwise difficult to synthesize.

Acknowledgments:

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12. Isobacteriochlorins **7**, **8** and bacteriochlorins **9**, **10** are obtained from purpurin-18 methyl ester (containing a fused anhydride ring). Thus, these compounds can also be named as isobacteriopurpurins and bacteriopurpurins.

13. NMR and Mass (FAB) data:

Isobacteriochlorin 2: 8.23, 7.33, 6.51 (each s, 1H, Meso H); 4.19 (ABX, 2H, $CH_2CO_2CH_3$); 3.99, 3.89, 3.77, 2.86, 2.64 (each s, 3H, 3 X CO_2CH_3 and 7, 12- pyrrolic ring CH_3); 3.84 and 3.40 (each m, 1H, 17-H and 18-H); 3.17 (q, 2H, 3-CH_2CH_3); 3.17 (q, 2H, 8-CH_2CH_3); 2.36 (m, 2H 17a-CH_2); 2.14 (m, 4H, 17a CH_2 and 3-CH_2CH_3); 1.80 (s, 3H, 18-CH_3(ring A); 1.38 (t, 3H, 8-CH_2CH_3); 1.30 (d, 3H, 18-CH_3); 0.70 [t, 3H, 3-CH_2CH_3 (ring A)]. Mass calculated for $C_{37}H_{44}N_4O_8Ni$: 730.2506. Found: 731 (M+1).

Isobacteriochlorin 3: 8.20, 7.2 and 6.72 (each s, 1H, MesoH); 4.20 (ABX, 2H, $CH_2CO_2CH_3$); 3.99, 3.68, 3.23, 2.84, 2.60 (each s, 3H, 3 X CO_2CH_3 and 2 X pyrrolic ring CH_3); 3.56 and 3.44 (each m, 17- H and 18 -H); 3.13 (q, 4H, 3 and 8- CH_2CH_3); 1.70-2.20 (m, 17a -CH_2 and 17b -CH_2); 1.40 (d merged with s, 18- CH_3 and 12- CH_3); 1.29 (6H, 2 X ring CH_2CH_3).Mass calculated for $C_{37}H_{44}N_4O_8Ni$: 730.2506. Found 731 (M+1).

Isobacteriochlorin 7: 7.91, 7.40, 6.82 (each s, 1H, Meso H); 4.09 , 3.59 (each m, 1H, 17-H and 18-H); 3.62 (s, 3H, CO_2CH_3); 3.04 (q, 2H, 8-CH_2CH_3); 2.68 , 2.53 (each s, 6H, 7, 12 -CH_3); 2.41 (m, 4H, 17a-CH_2 and 3-CH_2CH_3); 1.88 [s, 3H, 2-CH_3 (ring A)]; 1.79 (m, 2H, 17b-CH_2); 1.28 (m, 5H, 18-CH_3 and 8-CH_2CH_3); 0.74 [t, 3H, 3-CH_2CH_3(ring A)]. Mass calculated for $C_{34}H_{37}N_4O_7Ni$: 672.38. Found: 673 (M+1).

Isobacteriochlorin 8: 8.14, 7.34, 6.91 (each s, 1H, Meso H); 3.98 (m, 1H, 18-H); 3.52 (s, 3H, CO_2CH_3); 3.46 (m, 1H, 17-H); 3.15 (q, 2H, 3-CH_2CH_3); 2.96, 2.63 (each s, 3H, CH_3); 2.44 (m, 4H, 17a- CH_2, 8-CH_2CH_3); 1.78 (m, 2H, 17b-CH_2); 1.42 (m, 6H, 3, 8-CH_2CH_3); 1.34 (s, 3H, 12-CH_3); 1.09 (d, 3H, 18-CH_3). Mass calculated for $C_{34}H_{37}N_4O_7Ni$ 672.38. Found: 673 (M+1).

Zn (II) bacteriochlorin 9: Two sets of peaks were observed. 11.08 (as 2s, 1H, CHO); 9.44, 8.84, 8.43 (each as 2 s, each 1H, Meso H); 5.13 (m, 2H, 18-H); 4.18 (m, 2H, 17-H); 3.68 (s, 6H, 2 X CO_2CH_3); 3.42 (m, 12H, 2-CH_3 and 13-CH_3); 2.58 (m, 4H, 2 X 8-CH_2CH_3); 2.50, 2.12 (each m, 2H, 17a- and 17b-CH_2 espectively); 1.80 (2d, each 3H, 18-CH_3); 0.68 (2t, each 3H, 8-CH_2CH_3). Mass calculated for $C_{33}H_{34}N_4O_8Zn$: 676.86. Found: 677 (M+1), 659 (M+- OH), 641 (M+-2 OH).