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Remarkable Regioselective Position-10 Bromination of Bacteriopyropheophorbide-*a* and Ring-B Reduced Pyropheophorbide-*a*

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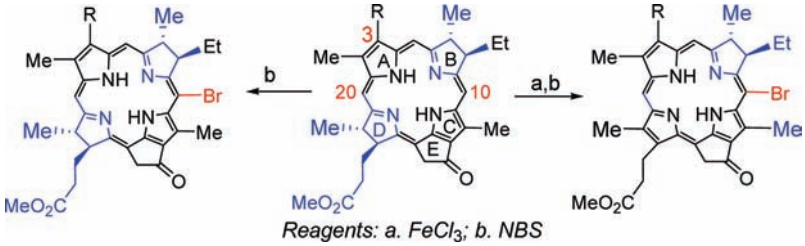
ABSTRACT



Both bacteriopyropheophorbide-*a* and ring-B reduced pyropheophorbide-*a* on reacting with NBS (*N*-bromosuccinamide) undergo electrophilic bromination to provide 10-bromo analogs. The electronic nature of the substituents present at position-3 did not make any difference in the regioselective outcome of the brominated products. These relatively stable brominated chlorins and bacteriochlorins provide an easy way of introducing a wide variety of functionalities, which could be extremely useful in developing improved agents for biomedical applications and supramolecular chemistry.

In recent years, chlorins and bacteriochlorins in which one or two pyrrole rings diagonal to each other are reduced have received enormous interest in applications to material science, engineering, biology, and medicine.[1,2] Continued efforts are also underway to synthesize a large number of supramolecular structures to understand the plant and bacterial photosynthetic reaction centers.[3,4] Due to long-wavelength absorption characteristics of both chlorins and bacteriochlorins, these chromophores have also generated immense interest in developing improved agents for tumor-imaging and phototherapy (PDT).[5]

For quite sometime, one of the main objectives of various laboratories has been to develop improved PDT and/or imaging agents with enhanced tumor specificity.[6,7]

In the development of such agents, the presence of multiple functionalities in the molecule could be advantageous as they provide additional opportunities to change the overall lipophilicity and target specificity by

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adding desired tumor-targeting moieties to the molecule.[8] Further, the presence of such a characteristic in the molecule could also help in developing a single agent for tumor imaging and therapy using a "See and Treat Approach".[9−11]

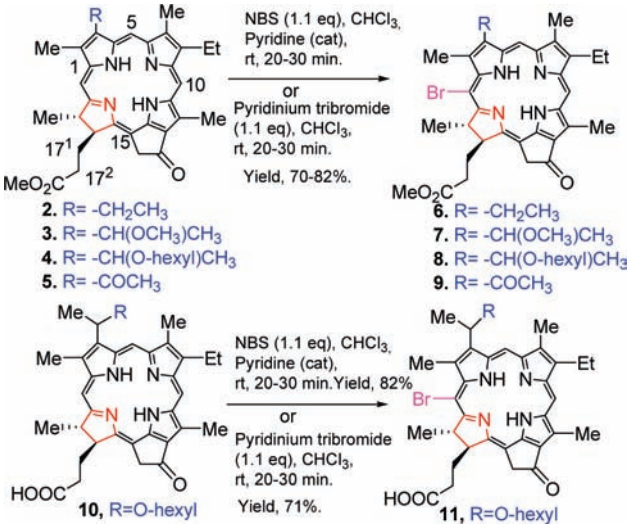
In porphyrin chemistry, halogenation reactions have been used extensively to functionalize porphyrin and chlorin systems.[12−14] Porphyrins can undergo fluorination, chlorination, bromination, and iodination, although the last reaction is considerably less favored because of steric and electronic factors. When both the *meso*- and β-halogenation of unsubstituted positions at the porphyrin periphery are possible, *meso*-chlorination is usually observed, whereas β-substitution is favored for both bromination and iodination.[1] The site of halogenation is significantly determined by the size and reactivity of the halogen. Since bromine is of an intermediate size among the halogens, *meso*-bromination is also observed.

In the chlorin system, *e.g.* methyl mesopyropheophorbide-*a*, the halogens can be selectively introduced at position-20.[15,16] For example, mesopyropheophorbide-*a* on reacting with either *N*-bromosuccinamide or pyridinium tribromide undergoes electrophilic bromination to give only the corresponding 20-bromo analogs due to high electron density at the *meso*-position next to the reduced ring. The other adjacent *meso*-position (position 15) is not available for any substitution because of its involvement in forming a fused isocyclic ring system (ring E). Substitution at the pyrrole periphery is also ruled out because all positions are occupied with alkyl or propionic ester functionalities. In recent years there has been considerable interest in using chlorophyll-*a* or bacteriochlorophyll-*a* based analogs to understand the photosynthetic reaction centers.[17,18] For the development of supramolecular structures, a variety of halogenated porphyrins, chlorins, and bacteriochlorins are being used as substrates.[19]

We have recently shown that bacteriopyropheophorbide-*a* on treated with appropriate oxidizing agents can be transformed into the corresponding ring-B or ring-D reduced chlorins in excellent yields.[20] In this report, we present a remarkable regioselective bromination of the ring-B reduced chlorins and bacteriochlorins derived from bacteriochlorophyll-*a*. Although the syntheses and photophysical properties of the brominated porphyrins and synthetic bacteriochlorins have been investigated,[21] comparatively little is known about the reactivity of naturally occurring chlorins and bacteriochlorins toward halogenation and functionalization.

To establish the reaction conditions for bromination in chlorin and bacteriochlorin systems, we started with methyl pyropheophorbide-*a*, and to avoid vinylic bromination or multiple bromination,[22] the methyl pyropheophorbide-*a* was metalated with $Zn(OAc)_2$. This was followed by reduction with Pd/C in THF and demetalation to give the 3-devinyl-3-ethyl pyropheophorbide-*a* (mesopyropheophorbide-*a*) **2**. It was then treated with NBS in chloroform in the presence of a catalytic amount of pyridine, to obtain the corresponding bromo analog **6**. We also employed other brominating agents; only pyridinium tribromide gave a similar yield and selectivity when

Scheme 1. Bromination of Pyropheophorbide (Ring-D Reduced) System



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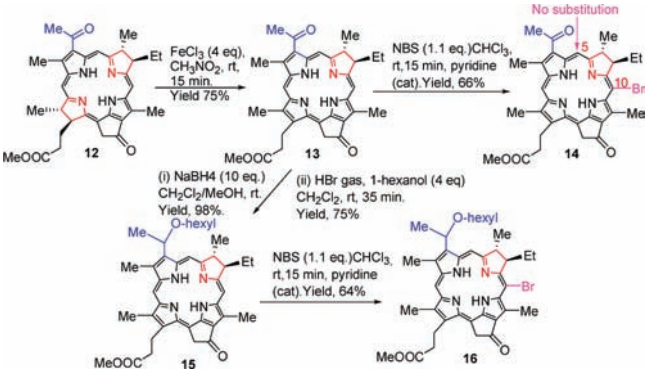
compared with the NBS reaction products. The 2D-NMR analysis of compound **6** indicated that the bromine was attached at position 20 of the macrocyclic ring, as reported in the literature. To investigate the effect of different functionalities at position 3, a series of compounds containing an alkyl ether or acetyl group **3**, **4**, and **5**, respectively, were synthesized which, upon bromination (NBS/$CHCl_3$/pyridine or pyridinium tribromide/$CHCl_3$), only gave the corresponding 20-brominated compounds **7**, **8**, and **9**, respectively (Scheme 1). These results suggest that the presence of electron-donating or -withdrawing (acetyl) groups at position 3 does not make any significant difference to the selectivity of halogenation. As expected, the presence of a free carboxylic acid at the 17^2-position (compound **10**), instead of a methyl ester, did not alter the position of the bromine in product **11**.

We next turned our attention to ring-B reduced chlorins **13** and **15** containing either an acetyl or hexyloxyethyl group at position 3, obtained by following the methodology recently developed in our laboratory.[20,23] Both chlorins on reacting with NBS or pyridinium bromide gave the corresponding 10-bromo analogs **14** and **16**, respectively. To our surprise, no substitution was observed at position 5 adjacent to reduced ring B (Scheme 2).

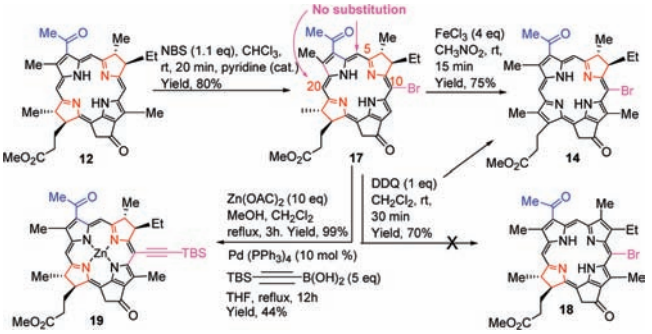
Scheme 2. Regioselective Bromination of Ring-B Reduced Chlorins



The selectivity of bromination reaction was then investigated in bacteriochlorin **12** containing both the ring-B and ring-D reduced pyrrole rings. We were expecting a mixture of 5-, 10-, and 20-brominated analogs; instead, only the 10-bromo-bacteriopyropheophorbide-*a* **17** was isolated as the sole product. Interestingly, oxidation of **17** on reacting with DDQ did not yield the expected ring-D reduced chlorin **18** but rather gave the unexpected ring-B reduced chlorin **14** (Scheme 3).

To exemplify the application of these brominated derivatives, we performed the Pd cross-coupling reaction (Suzuki coupling)[24] on 10-bromobacteriochlorin **17** by following the reaction sequences shown in Scheme 3 and the desired Zn(II) 10-silyl acetylene analog **19** was isolated in good yield, which could be converted into a series of novel supramolecular structures and these studies are currently in progress. The regioselective electrophilic substitution of a particular *meso*-position adjacent to the reduced ring of the chlorins and bacteriochlorins can be explained on the basis of its higher electron density.

Scheme 3. Synthesis of 10-Bromo-bacteriopyropheophorbide-*a*, 10-Bromo-ring-B Reduced Chlorin and the Corresponding 10-Silyl Analog



Density functional calculations were carried out to clarify the difference in reactivities for bromination at the 10- and 20-position. Tables 1 and 2 show the electronic charges of the starting materials and the Hartree energies of the brominated products obtained by DFT with the B3LYP/6-31G(d) basis set.[25] For compounds **2**–**5** and **10** shown in Scheme 1, the calculated electron densities at position 20 were higher than those at position 10 (Table 1).

Table 1. Calculated Electron Densities at Positions 5, 10, and 20 of Certain Chlorins and Bacteriochlorins[a]

	Position 20	Position 10	Position 5
Ring D reduced			
2	**−0.316**	−0.226	−0.248
3	**−0.318**	−0.229	−0.244
4	**−0.317**	−0.227	−0.241
5	**−0.303**	−0.233	−0.236
10	**−0.318**	−0.227	−0.240
Ring B reduced			
13	−0.223	−0.313	**--0.316**
15	−0.242	−0.311	**−0.316**
Rings B and D reduced			
12	−0.288	−0.298	**−0.302**

[a] Most negative values are presented by bold numbers.

In contrast, the ring-B reduced compounds **13**, **15** and the ring-B and -D reduced bacteriochlorin **12** exhibit a

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higher electron density at position 5. However, no bromination at the 5-position was observed even though the calculated electron density was the highest at this position. Perhaps, interference from adjacent substituents at the 3^1-position such as a carboxymethyl or hexyloxy group prevents bromination at the 5-position. This could be the reason for the higher stability of the 10-brominated isomer.

Table 2. Calculated Energies (in hartree[a]) of Certain Chlorins and Bacteriochlorins at Positions 5, 10, and 20[b]

	Position 20	Position 10	Position 5
Ring D reduced			
6	**−4334.3282435**	−4334.325446	−4334.3174495
7	**−4409.5330262**	−4409.5308662	−4409.5151262
8	**−4645.4141285**	−4645.4120693	−4645.3961645
9	**−4408.3363894**	−4408.3355856	−4408.3290654
11	**−4606.1070822**	−4606.1049602	−4606.0890733
Ring B reduced			
14	−4408.3244631	**−4408.3393993**	−4408.3328343
16	−4645.4012942	**−4645.4144882**	−4645.4006457
Rings B and D reduced			
17	−4409.5395198	**−4409.5458701**	−4409.5387054

[a] 1 hartree = 629.51 kcal mol^{-1}. [b] Most stable energy values are presented by bold numbers.

The Hartree energies in Table 2 suggest that 20-brominated ring-D reduced compounds, **6**–**9** and **11**, are the most stable isomers. Thus, bromination favors the 20-position rather than the 5- or 10-position. On the other hand with B-ring reduced chlorins (**13** and **15**) and bacteriochlorin (**12**), bromination takes place at the 10-position which has the lowest Hartree energy among these isomers.

The electronic absorption spectra of key products ring-D reduced 2-bromo analog **9**, its isomer ring-B reduced 20-bromo analog **14**, and the methyl 20-bromo-bacteriopyropheophorbide-*a* **17** are shown in Figure 1. As can be seen, the electronic absorption spectra of both chlorins **9** and **14** were almost identical. The Soret band exhibited strong absorption at 416 nm, and the long wavelength absorption was observed at 686 and 691 nm respectively. As expected bacteriochlorin **17** showed longer wavelength absorption at 365 nm and the Soret band was observed at 746 nm. Thus compared to chlorin **9** and **14** a blue shift of 55 nm was observed. The spectra were measured at equimolar concentrations in dichloromethane.

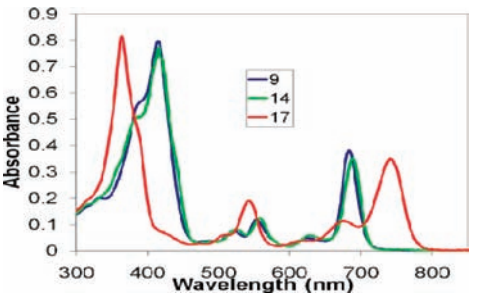

Figure 1. Electronic absorption spectra of methyl 3- acetyl-20-bromo-3-devinyl pyropheophorbide-*a* **9**, 3-acetyl-10-bromo-ring-B reduced chlorin **14** and the methyl 3-acetyl-10-bromo-bacteriopyropheophorbide-*a* **17** in dichloromethane.

In summary, we report herein a facile approach for introducing bromo- functionality at position 10 or 20 of certain chlorins and bacteriochlorins. The regioselective approach provides an opportunity to develop a variety of novel supramolecular structures for biomedical applications and to understand more about the photosynthetic reaction center, especially the reasons for the selection of ring-D reduced chlorins (chlorophyll-*a*) over the corresponding ring-B reduced isomer by nature.

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Supporting Information Available. Experimental procedures, ^1H and ^{13}C NMR spectra of new chlorins and bacteriochlorins. This material is available free of charge via the Internet at http://pubs.acs.org.