Relative Stereochemistry of the A Ring of Plant Bile Pigments

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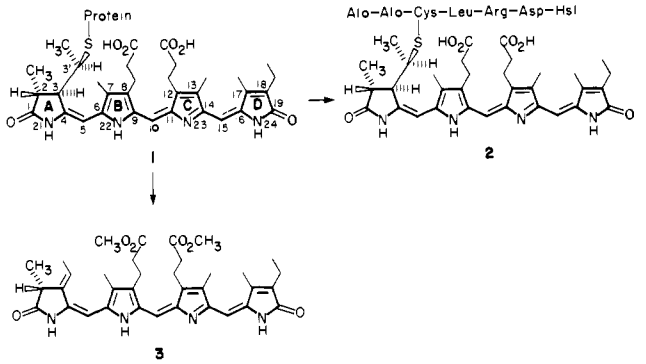
Abstract: The synthesis and characterization, including the stereochemistry, of a series of 3,4-dihydropyrromethenones and 2,3-dihydrodioxobilins are described. High-resolution ^{1}H NMR spectral analysis allows the determination of the A ring coupling constants for a series of cis and trans model compounds. From these data and correlations, the relative stereochemistry in the A ring of phycocyanin and similar bile pigment structures can be concluded.

Complete structure elucidation of plant bile pigments (**1**) has been limited by the common practice of cleaving the linear tetrapyrrole (**3**) from its covalently bound protein.[1-6] This process

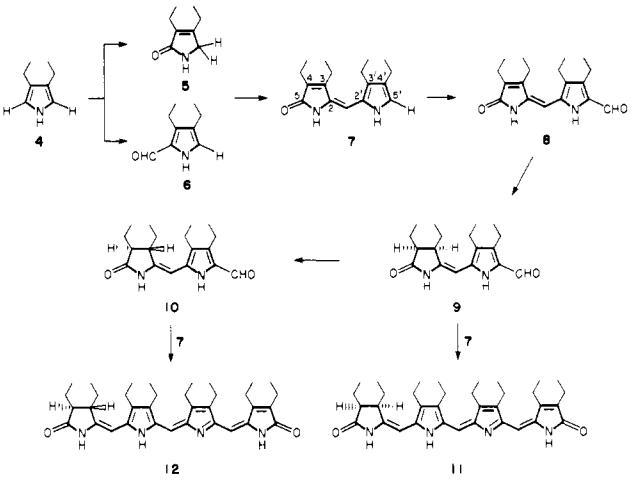


has been a valuable tool for gross structural determination, as the resulting product can be easily studied by spectroscopy,[7] chemical degradation,[8] and total synthesis.[9,10] The approach, however, suffers from several disadvantages. Firstly, all direct information on the chromophore–protein linkage, including two stereo centers, is lost.[1] Secondly, side reactions leading to complex mixtures of unnatural chromophores have been observed.[11] Finally, the yield in the cleavage step is frequently low.[4,5] These limitations, when combined with the fact that complex proteins are involved containing multiple chromophores with possible structural variations, indicate a more controlled method for structural study is necessary.

In previous studies, we have developed methodology to conclusively ascertain the nature of the protein–tetrapyrrole covalent bond.[12-14] By mild, selective degradation of the protein moiety without alteration of the chromophore or its attachment site, the three chiral centers about the A ring of the bile pigment are retained. Therefore, we are in a unique position to study the stereochemistry at these centers. For simplicity, we have chosen to concentrate on the phycocyanin series of bile pigments, as it contains only three chiral centers, all contiguous about the A ring.

The proposed structure for the peptide pigment from the N-terminal portion of the β-subunit of *C*-phycocyanin from *Synechococcus* sp. 6301 is shown in structure **2**.[12] Assignment of stereochemistry at C-2 is based on the report that chromic acid oxidation of *C*-phycocyanin results in the formation of (*E*)-2-ethylidene-3(*R*)-methylsuccinimide.[15] Unfortunately, the actual analytical and spectroscopic data for the compound obtained from the bile pigment degradation are unavailable in the literature. Also, *C*-phycocyanin contains three phycocyanobilins, each with possible structural differences.[16] Although it seems reasonable that the three chromophores have the same stereochemistry, until this determination is made for each chromophoric unit, the question remains unanswered.

Scheme I. Synthesis of *cis*- and *trans*-2,3-Dihydrooctaethylbiliverdins 11 and 12



Assignment of the relative stereochemistry between C-3 and C-3′ also is based on degradative evidence. Mechanistic interpretations indicate that an antiperiplanar elimination of the sulfone resulting from thioether oxidation gives the observed (*E*)-ethylidenesuccinimide.[17] Although the findings have been corraborated by thiol elimination to the same product on numerous occasions, no detailed mechanistic studies on this reaction have been done.[15] Despite the evidence presented, the multiple chromophore problem casts doubts on the utility of these data.

The remaining stereochemical relationship to be addressed, then, is the relative stereochemistry between the C-2 and C-3 centers. Having two adjacent protons in a rigid five-membered ring suggests as a solution the homonuclear coupling constant determination of the dihedral angle relationship. Conclusions would require the proper model compounds, as the system is too complex to approach theoretically.[18] Although some model systems are available, the information cannot be directly related to the natural product.[17,19] Possible solvent effects and complicating side-chain

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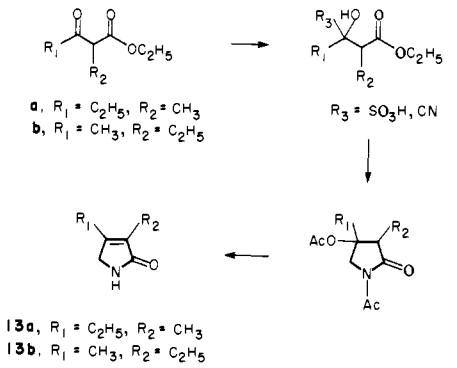
Scheme II. Synthesis of Ethylmethylpyrrolinones 13a and 13b



functionality have prevented a definitive interpretation.

In this report, we present a systematic study of model 2,3-dihydrodioxobilins and correlate their NMR properties with those of natural bile pigments. The information gained, if used with properly collected degradative data, should allow the complete absolute stereochemical assignment of any phycocyanobilipeptide.

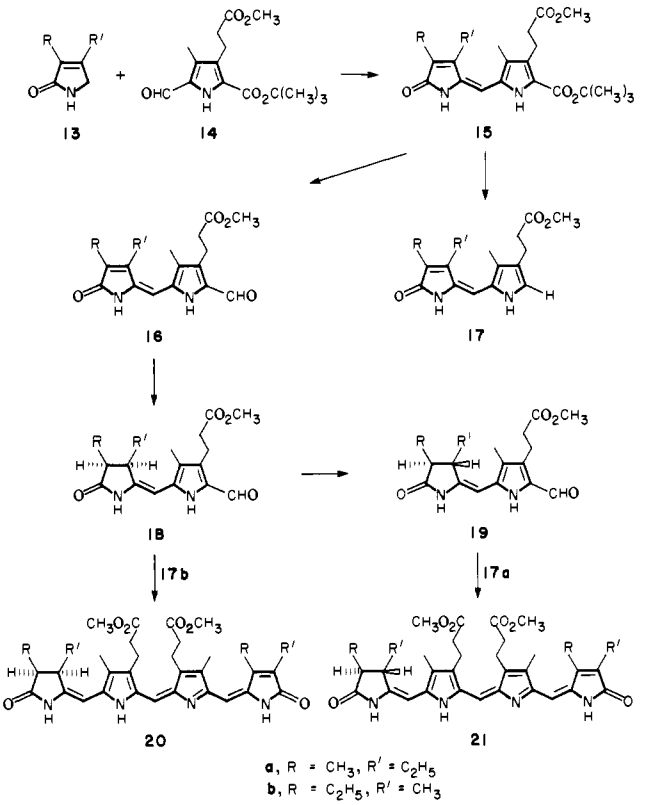
Results and Discussion

Synthesis of *cis*- and *trans*-2,3-Dihydrooctaethylbiliverdin. Our initial synthetic goal was to gain entry into symmetrically substituted octaalkyldihydrobiliverdins (Scheme I). The octaethyl series was chosen to avoid any solubility problems that might restrict the analysis. We began with the 1,3-dipolar addition of the tosylmethyl isocyanide anion to an α,β-unsaturated ketone that allows for rapid and facile entry into 3,4-dialkylpyrroles after reduction of the carbonyl group.[20,21] The 3,4-diethylpyrrole (**4**) thus obtained served as the starting material for all four pyrrolic rings. This pyrrole could be formylated under Vilsmeier conditions to give an appropriately functionalized unit **6** for the B and C rings.[22] The diethylpyrrole could also be oxidized in a stepwise manner, first to give 3,4-diethyl-3-pyrrolin-2-one (**5**) and then further to give 2,3-diethylmaleimide.[22,23] The pyrrolinone was the A and D ring precursor, while the maleimide was a necessary compound for the structure proof.

Base-catalyzed condensation of pyrrolinone **5** and formylpyrrole **6** gave 3,3′,4,4′-tetraethyl-5(1*H*)-pyrromethenone (**7**), again a common intermediate.[22] Formylation, this time with trimethyl orthoformate, gave the key pyrromethenone **8** into which the 3,4-dihydro feature could be introduced.[10] This catalytic reduction of the 5′-formylpyrromethenone **8** gave a mixture of products reduced at the 3,4 and the methine double bonds, as well as the tetrahydro material.[24,25] The desired 3,4-dihydro compound was the major product and could be easily separated by chromatography. *cis*-3,4-Dihydro-5′-formylpyrromethenone (**9**) obtained from the catalytic reduction was epimerized completely to the trans isomer by refluxing in methanolic sodium hydroxide.[24] Operationally, it is easier to carry out the epimerization on the crude reduction mixture and then do a single purification to obtain the trans compound.

The final synthetic transformation in this series was to couple the aldehydic pyrromethenones **9** and **10** with the 5′(1*H*)-pyrromethenone **7** to give the dioxotetrapyrroles **11** and **12** without epimerization at either C-2 or C-3.[26] A final HPLC purification of the resulting product showed that no epimerization had occurred, especially if one uses only 33 mol % of $POBr_3$ and 100 mol% of 2,5-di-*tert*-butyl-4-methylpyridine for the condensation reaction.

Scheme III. Synthesis of 2,3-Dihydrodioxobilins 20a, 20b, 21a, and 21b



Synthesis of 2,3-Dihydro Bile Pigments. Although the octaalkyl series gave us a valid 2,3-dihydro model system, a more appropriately functionalized linear tetrapyrrole was necessary for direct correlation with the natural pigments. Due to the inherent dissymmetry of the bile pigments, a more elaborate synthetic scheme to the ring precursors was required. However, the synthesis is still quite convergent, and the key synthetic transformations to obtain the 2,3-dihydro feature and to couple to the ultimate dioxotetrapyrroles were retained.

The necessary pyrrolinones **13a** and **13b** were synthesized through their corresponding cyanohydrins (Scheme II).[27] We found that the intermediacy of the bisulfite adduct avoided the need for large amounts of anhydrous HCN.[28] Attempts at reducing the cyanohydrin with platinum failed; however, the now standard Raney nickel reduction led smoothly to the desired compounds.[27]

The formylpyrrole **14** came from an initial Knorr condensation followed by extensive functional group transformations to obtain the necessary substituent pattern.[29,30] Base-catalyzed condensation to pyrromethenones **15** was done in methanolic KOH as in the ethyl series (Scheme III).[30] This required a subsequent reesterification with diazomethane. Attempts to carry out the condensation with sodium methoxide resulted in significantly lower yields.

The 5′-*tert*-butyloxycarbonyl group served as a stabilizing group during the pyrrole synthesis. Although catalytic reduction to the dihydro series can be done on the ester,[24] we wanted to minimize the number of transformations after the reduction in order to avoid exposure to epimerization. Therefore, an acid-catalyzed decarboxylative formylation was used and gave the 5′-formylpyrromethenone **16** directly. On the other hand, treatment of the *tert*-butyl ester with neat trifluoroacetic acid for 5 min allows one to isolate the 5′-acid in good yield, while a longer reaction time

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(2 h) effects decarboxylation to give the 5′(1*H*)-pyrromethenone **17**. This latter compound is the precursor of the C,D-ring moiety.

The remainder of the synthesis was directly analogous to the ethyl series and led to the synthesis of the four 2,3-dihydrodioxobilins **20a**, **20b**, **21a**, and **21b** as shown in Scheme III. The side-chain methyl ester introduced some problems, as some hydrolysis was seen in both the epimerizing and coupling steps. However, in both cases the acid could easily be separated from the ester and reesterified with stoichiometric diazomethane. The final products were purified by HPLC, and again the coupling was effected without epimerization.

Degradative Structure Proofs. It has always been assumed that catalytic hydrogenation gives cis hydrogen addition to the pyrromethenones. However, the empirical nature of the experimental results makes such a simplistic assumption tenuous. Since the methine double bond is trisubstituted while the 3,4-double bond is tetrasubstituted, it is surprising that the 3,4-dihydro compound is the major product. Furthermore, if the reduction is done under alkaline conditions or with other catalysts, only the pyrromethane is seen.[24] Due to uncertain mechanistic paths in such a complex system, we concluded that a more rigorous stereochemical proof was required in our dioxobilin series.

Diethylmaleimide and ethylmethylmaleimide were independently synthesized by oxidation of the corresponding pyrroles.[22,23] Catalytic hydrogenation of these compounds over PtO_2 gives the *cis*-succinimides in nearly quantitative yield.[31] The *trans*-succimimides were then obtainable from a strong base-catalyzed epimerization.

Each of the dioxobilins was then subjected to the now standard chromic acid oxidation.[32] In the octaethyl series, diethylmaleimide was generated from the B, C, and D rings. From the *trans*-tetrapyrrole, only the *trans*-succinimide resulting from A ring oxidation was seen by GC analysis; no *cis*-succinimide was present. The *cis*-tetrapyrrole likewise gave diethylmaleimide and only *cis*-diethylsuccinimide. Although no rigorous detection limits were established, less than 10% of the isomeric succinimide would easily have been seen in each case.

The bile pigment degradation products are more easily observed by TLC analysis, using the sensitive chlorine/*o*-anisidine reagent for visualization.[33,34] Chromic acid oxidation of each of the four compounds **20a**, **20b**, **21a**, and **21b** gave poor yields, as usual, but no interfering side products. Each dioxotetrapyrrole gave positive tests for ethylmethylmaleimide and hematinic acid methyl ester, resulting from the B, C, and D rings. From both *trans*-tetrapyrroles **21a** and **21b** only *trans*-ethylmethylsuccinimide was obtained. The *cis*-tetrapyrroles **20a** and **20b** gave *cis*-ethylmethylsuccinimide with a barely detectable trace of the *trans*-succinimide. Considering the sensitivity of the visualization process, the trace amounts are extremely small. Since the dioxotetrapyrroles **20a** and **20b**, having been purified by preparative HPLC prior to degradation, were free from contaminating trans-isomer, this indicates that a small amount of epimerization did occur in the oxidation step.[35] The results, however, leave no doubt that the correct cis and trans assignments have been made.

^{1}H NMR Analysis. The ^{1}H NMR assignments for the *cis*- and *trans*-3,4-dihydro-5′-formylpyrromethenones **18a**, **18b**, **19a**, and **19b** in $CDCl_3$ are shown in Table I. Decoupling experiments allowed us to assign all proton resonances and to determine the 3-H/4-H coupling constants (Table IV) despite a few overlapping resonances. This analysis greatly facilitated the interpretation of the more complicated tetrapyrrole spectra.

The ^{1}H NMR assignments for the dioxotetrapyrroles **20** and **21** in $CDCl_3$, along with shifts reported in a previous study,[25] are found in Table II. Compound **21b** is included in this study for completeness, as we believe that conclusive evidence for all bile pigment attachments through the A ring has yet to be presented.

Table I. ^{1}H NMR Assignments for the 3,4-Dihydro-5′-formyl *cis*-18 and *trans*-19 Pyrromethenones in $CDCl_3$

assignment	chemical shift, ppm			
	18a (cis)	18b (cis)	19a (trans)	19b (trans)
3- or 4-CH_2CH_3	1.01	1.05	1.02	1.03
3- or 4-CH_3	1.21	1.23	1.32	1.36
3- or 4-CH_2CH_3	1.6–1.8	1.5–2.0	1.5–2.0	1.5–2.0
3′-CH_3	2.03	2.02	2.02	2.03
4-H	2.79	3.05	2.42	2.17
4′-$CH_2CH_2CO_2CH_3$	2.58	2.57	2.58	2.58
4′-$CH_2CH_2CO_2CH_3$	3.06	3.10	3.06	3.06
3-H	2.95	3.26	2.65	2.85
4′-$CH_2CH_2CO_2CH_3$	3.68	3.68	3.68	3.67
meso-H	5.37	5.35	5.31	5.31
5′-CHO	9.54	9.54	9.54	9.53

Our values agree well (±0.05 ppm) with those reported[25] with the exception of the C-2-H, C-2-CH_3, and C-3-H. These resonances show the most variation when the pyrromethenones and the dioxotetrapyrroles are compared as well. The decoupling experiments, though, leave no doubt about the assignments made. The chemical shift variation may reflect differing protonation states due to trace amounts of acid in the solvent or concentration effects, as these molecules are known to exhibit complex solution conformational behavior.

In our study of the β_1-phycocyanobiliheptapeptide,[12] we found pyridine to be an excellent solvent for avoiding aggregation and conformational effects. The basicity of the pyridine, present in large excess, precludes differential protonation of the substrate, and the aromatic nature of this solvent encourages intercalation between substrate molecules, preventing aggregation. Thus, the conformations adopted by the dioxotetrapyrroles appear to be uniform.

To complete our present study, therefore, the 360-MHz ^{1}H NMR spectra of the dioxobilins were recorded in pyridine-d_5 (Table III). Not only does this serve to avoid the problems mentioned above, but it also allows us to make a direct comparison with previous natural products studied in pyridine. There is much less variation in resonances for the model compounds, and several patterns appear. The C-15-H in the 2-ethyl-3-methyl series is shifted downfield approximately 0.1 ppm from that in the 3-ethyl-2-methyl series. The A-ring methyl group for the former series is also shifted downfield 0.1 ppm. Finally, although the C-2-H and C-3-H still show the most variation in chemical shift, the 2-ethyl-3-methyl cis and trans compounds show a significant (0.2–0.3 ppm) upfield shift of the C-2-H as compared to the corresponding 3-ethyl-2-methyl compounds.

That our models truly reflect the actual case is shown by the excellent agreement between the shifts for **21a**, the most likely model, and the β_1-phycocyanobiliheptapeptide (**2**). Where minimal perturbation of structure is seen, the agreement is within 0.05 ppm. Of particular importance is the correlation of the C-2-CH_3 and even the C-2-H. The substituents on the B, C, and D rings likewise agree well, although the presence of the acid rather than the ester causes slightly larger shift differences in the propionic acid resonances. The methine protons also show a substantial difference in shift, as has been seen before when comparing a free dioxotetrapyrrole to one which is peptide bound.[12] The nature of this difference is still unknown and, to be deciphered, will require synthesis of a more appropriate model incorporating the amino acid thioether moiety. The presence of the thioether likewise destroys any possible comparison at the C-3-H or any of the C-3-ethyl protons.

The most important stereochemical information comes from the pattern of A-ring coupling constants seen in Table IV. The $J_{3,4}$ for the functionalized *cis*-dihydropyrromethenones **18** centers around 8.1 Hz in $CDCl_3$ while the trans compounds **19** show $J_{3,4}$ ~5.0 Hz. The ethyl compounds show coupling constants quite a bit lower (6.8 and 2.9 Hz, respectively) due to the steric interaction between the C-3 and C-3′ ethyl groups which must distort the conformation. The dioxotetrapyrroles show slightly higher

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Table II. ^{1}H NMR Assignments for the 2,3-Dihydrodioxotetrapyrroles 20 and 21 in $CDCl_3$

assignment	chemical shift, ppm[a] 20a (cis)	20b (cis)	21a (trans)	22b (trans)
2- or 3-CH_2CH_3	1.01 (1.06)	1.02 (0.99)	1.06	0.99 (0.99)
17- or 18-CH_2CH_3	1.10 (1.10)	1.22 (1.22)	1.10	1.22 (1.22)
2- or 3-CH_3	1.12 (1.24)	1.27 (1.42)	1.24	1.43 (1.43)
2- or 3-CH_2CH_3	1.5-2.0 (1.7-2.0)	1.4-2.0 (1.6-1.8)	1.6-2.0	1.5-1.9 (1.6-1.8)
7-,13-,17-, or 18-CH_3	2.02 (2.02)	1.85 (1.84)	2.01	1.85 (1.85)
	2.11 (2.12)	2.02 (2.02)	2.11	2.02 (2.02)
	2.12 (2.12)	2.12 (2.12)	2.12	2.12 (2.12)
17- or 18-CH_2CH_3	2.32 (2.32)	2.55[b] (2.54)	2.32	2.55[b] (2.54)
2-H	2.85 (2.32)	2.6[b] (2.1)	2.35[b]	2.15[b] (2.92)
8,12-$CH_2CH_2CO_2CH_3$	2.54 (2.54)	2.54 (2.54)	2.54	2.54 (2.54)
	2.55 (2.55)	2.55 (2.54)	2.55	2.56 (2.54)
3-H	3.14 (2.75)	3.38 (2.93)	2.79	2.94 (2.14)
8,12-$CH_2CH_2CO_2CH_3$	2.90 (2.90)	2.90 (2.93)	2.90	2.90 (2.92)
	2.94 (2.94)	2.94 (2.93)	2.94	2.95 (2.92)
8,12-$CH_2CH_2CO_2CH_3$	3.66 (3.67)	3.67 (3.67)	3.67	3.67 (3.67)
	3.68 (3.68)	3.68 (3.68)	3.68	3.68 (3.68)
5-H	5.49 (5.47)	5.47 (5.47)	5.46	5.47 (5.48)
15-H	5.98 (5.98)	6.00 (6.01)	5.98	6.01 (6.01)
10-H	6.65 (6.64)	6.64 (6.65)	6.64	6.65 (6.66)

[a] Chemical shifts in parentheses are from ref 25. [b] Multiple resonances occur at these frequencies, but assignments can be made from decoupling and integration studies.

Table III. ^{1}H NMR Assignments for the 2,3-Dihydrodioxotetrapyrroles 20 and 21 in Pyridine-*d*

assignment	chemical shift, ppm 20a (cis)	20b (cis)	21a (trans)[a]	21b (trans)
2- or 3-CH_2CH_3	0.97	1.08	1.03 (1.48)[d]	1.03
17- or 18-CH_2CH_3	1.30	1.24	1.28 (1.23)	1.24
2- or 3-CH_3	1.24	1.33	1.36 (1.39)	1.47
2- or 3-CH_2CH_3	1.6-1.7	1.4-1.7	1.6-2.0[d]	1.6-2.0
7-,13-,17-, or 18-CH_3	1.95	1.94	1.96 (2.02)	1.96
	2.09	2.05	2.09 (2.07)	2.05
	2.11	2.10	2.11 (2.12)	2.11
17- or 18-CH_2CH_3	2.52	2.55	2.51 (2.48)	2.55
2-H	3.03[b]	2.71	2.62 (2.70)	2.40
8,12-$CH_2CH_2CO_2CH_3$	2.69	2.68	2.69 (2.83)[e]	2.69
	2.72	2.72	2.71 (2.85)[e]	2.72
3-H	*c*	3.28	2.7[b] (3.15)[d]	2.93
8,12-$CH_2CH_2CO_2CH_3$	3.00	3.00	3.00 (3.09)[e]	3.00
	3.09	3.08	3.09 (3.17)[e]	3.09
8,12-$CH_2CH_2CO_2CH_3$	3.62	3.62	3.62	3.62
	3.64	3.64	3.64	3.64
5-H	5.47	5.47	5.44 (5.87)	5.47
15-H	6.07	6.15	6.07 (6.08)	6.17
10-H	7.09	7.07	7.08 (7.29)	7.09

[a] Chemical shifts in parentheses are for the β_1-phycocyanobiliheptapeptide 2 from ref 12. [b] Multiple resonances occur at these frequencies, but assignments can be made from decoupling and integration studies. [c] Assignment could not be made due to obscurring resonances. [d] This chemical shift is not strictly analogous due to the presence of the thioether. [e] This chemical shift is not strictly analogous as compound 2 is present as the acid.

Table IV. Coupling Constants[a] Observed for Ring A

compd	stereochemistry	*J*, Hz ($CDCl_3$)	*J*, Hz (pyridine-d_5)
18a	cis	8.2	
18b	cis	8.0	
9	cis	6.8	
20a	cis	8.5	8.2
20b	cis	8.2	8.5
11	cis	6.5	7.6
19a	trans	4.9	
19b	trans	5.1	
10	trans	2.9	
21a	trans	5.4	5.7
21b	trans	6.0	5.8
12	trans	3.7	4.1

[a] These values represent the 3-H/4-H coupling constant for the 3,4-dihydropyrromethenones and the 2-H/3-H coupling constant for the 2,3-dihydrodioxotetrapyrroles.

J values, as now the tendency to form helical conformations removes some of the C-3/C-7 steric interaction. In pyridine, the coupling constants show the same behavior. For the *cis*-dioxotetrapyrroles **20** one sees $J_{2,3} \sim 8.3$ Hz while the trans compounds **21** exhibit $J_{2,3} \sim 5.8$ Hz. The coupling constants are not nearly as sensitive to solvent effects as are the chemical shifts. Again, as witnessed by the ethyl series, steric bulk about the C-3 and C-7 centers causes a pronounced (1.0–1.5 Hz) lowering of the coupling constant.

The pattern is clear. On the basis of the models with the appropriate substitution, **20a** and **21a**, one expects the 2-H/3-H coupling constant in pyridine to be 8.2 Hz in the cis configuration and 5.7 Hz in the trans configuration. Returning to the β_1-phycocyanobiliheptapeptide, we observe a coupling constant of 5.0 Hz for these protons.[12] As the steric effect of the thioether undoubtedly causes a slight lowering of the coupling constant, we can now conclusively state that this bile pigment has a trans relationship between the C-2-H and the C-3-H. Given the previously mentioned caveats in regard to the assignment of the stereochemistry at C-2 and C-3′, our results also can serve to assign the absolute stereochemistry at the C-2, C-3, and C-3′ centers as *R*,*R*,*R*.

These data are applicable to the phytochrome structure as well, as the pigment structure differs only in the substitution of a vinyl for an ethyl group at C-18. The chemical shift correspondence substantiates this claim.[13] Again, a coupling constant of 5.0 Hz was seen for the 2-H/3-H interaction. By direct analogy, a *trans*-dihydro A ring must be present. This completes the relative stereochemical assignment of the phytochrome tetrapyrrole. The absolute stereochemistry has yet to be determined; therefore, the possibilities for the C-2, C-3, and C-3′ centers remain *R*, *R*, *R* or *S*, *S*, *S*.

Although drawing the analogy to the phycoerythrin as well as other bile pigment series would seem straightforward, it is clear that the stereochemistry at C-16 has a profound effect on the spectroscopic properties of this chromophore.[36] In our studies in this series, we found a 2-H/3-H coupling constant of 3.5 Hz in D_2O.[14] This low value is the first good evidence for the trans configuration in that series. However, because of possible solvent differences and until the phycoerythrin model series is synthesized, no stronger argument can be made.

Experimental Section

General Methods. Tetrahydrofuran (THF) was distilled from sodium/benzophenone, methanol was distilled from magnesium, methylene

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chloride was distilled from phosphorus pentoxide, *tert*-butyl alcohol and ethyl acetate were distilled from calcium hydride, pyridine was distilled from *p*-toluenesulfonyl chloride and then from calcium hydride, and trimethyl orthoformate was freshly distilled before use. Potassium *tert*-butoxide was freshly sublimed before use. Sodium methoxide was prepared immediately before use, and all other reagents were made as referenced or used directly from commercial suppliers after verification.

Melting points were measured on a Büchi capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 137 spectrophotometer with polystyrene film for calibration (1601.4-cm^{-1} absorption). UV/Vis spectra were recorded in methanol with a Perkin-Elmer 522A spectrophotometer. Unless otherwise noted, the ^1H NMR spectra were determined on the UCB-200 spectrometer (a homemade FT instrument operating at 201.95 MHz). The ^1H NMR spectra in pyridine-d_5 were recorded on the University of California, Davis, 360-MHz NMR spectrometer. All chemical shifts are expressed in parts per million (δ) downfield from an internal Me_4Si standard. The tabulated chemical shifts were recorded on approximately 15 mM samples. Multiplicities are not included for the sake of clarity, but are easily inferred from the data given. High-resolution (exact mass) mass spectra were obtained on a Kratos MS-50. Elemental analyses were performed by the Analytical Laboratory, College of Chemistry, University of California, Berkeley.

Gas chromatography (GC) was done with a Hewlett-Packard 402 gas chromatograph with a He flow rate of 80–100 mL/min on a 1.8-m 5% Dexsil 300 on 90/100 Anakrom Q column (6 mm i.d.) operating at 160 °C. High-pressure liquid chromatography (HPLC) was done on an Altex analytical system consisting of two Model 110A pumps, a 155-10 UV Vis detector, and a Model 420 microprocessor controller/programmer. An Altex 10 × 250 mm, 10 μm Lichrosorb Si60 silica gel column was used with detection at 340 nm with the following solvent compositions (v/v): (A) 60% isooctane/40% ether; (B) 0.75% methanol/chloroform; (C) 1.25% methanol/chloroform; (D) 55% ether/45% hexane. Analytical thin-layer chromatography (TLC) was done on aluminum-backed silica plates activated at 100 °C for 2 h. A 3-fold multiple development was done by using the solvent system chloroform/ethyl acetate/cyclohexane (32:9:1)[37] and the R_F values recorded are after three developments. Visualization was by a modification of the chlorine/benzidine procedure,[33,34] substituting *o*-anisidine for benzidine.

Unless otherwise noted, all reactions were conducted under a nitrogen atmosphere. Evaporations were done on a Berkeley rotary evaporator after drying over Na_2SO_4, using a water aspirator followed by static evaporation with an oil pump.

3,3′,4,4′-Tetraethyl-5′-formyl-5(1*H*)-pyrromethenone (8). 3,3′,4,4′-Tetraethyl-5(1*H*)-pyrromethenone (**7**, 300 mg, 1.1 mmol)[22] was dissolved in 6 mL of trifluoroacetic acid at room temperature, trimethyl orthoformate (3 mL, 27 mmol) was added at once, and the solution was stirred for 5 min at room temperature. Rapid addition of 40 mL of ice water was followed by extraction with CH_2Cl_2 (4 × 10 mL), and the combined organic layer was dried and evaporated. The residue was recrystallized from 50 mL of methanol to give 302 mg (91%) of **8** as yellow needles: mp 194–195 °C; UV/vis λ_{max} (log ϵ) 415 (sh), 394 (3.94), 268 (3.94), 161 nm (sh); NMR ($CDCl_3$) δ 1.1–1.3 (4 × t, 12 H, 4 × CH_3), 2.43 (q, 2 H, CH_2CH_3, J = 7.5 Hz), 2.57 (q, 4 H, 2 × CH_2CH_3, J = 7.5 Hz), 2.78 (q, 2H, CH_2CH_3, J = 7.6 Hz), 6.10 (s, 1 H, meso-H), 9.60 (s, H, CHO), 10.77 (s, 1 H, NH), 10.85 (s, 1 H, NH). Anal. Calcd for $C_{18}H_{24}N_2O_2$: C, 72.0; H, 8.0; N, 9.3. Found: C, 72.3; H, 8.0; N, 9.4.

***cis*-3,4-Dihydro-5′-formyl-3,3′,4,4′-tetraethyl-5(1*H*)-pyrromethenone (9).** Pyrromethenone **8** (200 mg, 0.67 mmol) was suspended in 50 mL of methanol, 200 mg of 3% $PdCl_2/SrCO_3$[38] was added, and 1 atm of hydrogen was applied for 4 h or until the solution turned from yellow to colorless. Filtration and evaporation left a residue which was purified by preparative HPLC: R_t (solvent A, 4.2 mL/min) 8.4 min, dihydropyrromethanone 28.8 min, pyrromethanone 42.0 min. The product **9** (104 mg, 52%) was a slightly yellow solid and was recrystallized from methanol: mp 166–170 °C; UV/vis λ_{max} (log ϵ) 360 (4.10), 242 nm (4.16); NMR ($CDCl_3$) δ 1.00, 1.04, 1.11, 1.23 (4 × t, 12H, 4 × CH_3, 4 × J = 7.6 Hz), 1.4–1.7 (m, 2 H, $CHCH_2CH_3$), 1.8–1.95 (m, 2 H, $CHCH_2CH_3$), 2.47 (q, 2 H, CH_2CH_3, J = 7.6 Hz), 2.5–2.65 (m, 1 H, 4-H), 2.73 (q, 2 H, CH_2CH_3, J = 7.6 Hz), 2.9–3.0 (m, 1 H, 3-H), 5.38 (s, 1 H, meso-H), 9.51 (s, 1 H, CHO). Anal. Calcd for $C_{18}H_{26}N_2O_2$: C, 71.5; H, 8.7; N, 9.3. Found: C, 71.6; H, 8.7; N, 9.2.

***trans*-3,4-Dihydro-5′-formyl-3,3′,4,4′-tetraethyl-5(1*H*)-pyrromethenone (10).** The cis compound **9** (100 mg, 0.33 mmol) was dissolved in 40 mL of 1 N NaOH in MeOH and refluxed for 2.5 h. The methanol was evaporated, 20 mL of 1 M aqueous H_3PO_4 was added, the aqueous solution was extracted with CH_2Cl_2 (4 × 10 mL), and the combined CH_2Cl_2 solution was washed with 10 mL each of H_2O and saturated aqueous NaCl and dried. After filtration and solvent evaporation, the residue was purified by HPLC as described for cis compound **9** to give **10** as a slightly eyllow solid (76 mg, 76%): mp 181–185 °C; UV/vis λ_{max} (log ϵ) 366 (4.09), 239 nm (4.14); NMR ($CDCl_3$) δ 0.99, 1.01, 1.11, 1.23 (4 × t, 12 H, 4 × CH_3, 4 × J = 7.5 Hz), 1.6–1.9 (m, 4 H, 2 × $CHCH_2CH_3$), 2.25–2.35 (m, 1 H, 4-H), 2.46 (q, 2 H, CH_2CH_3, J = 7.6 Hz), 2.73 (q over m, 3 H, CH_2CH_3 and 3-H, J_q = 7.6 Hz), 5.32 (s, 1 H, meso-H), 9.48 (s, 1 H, CHO), 10.92 (s, 1 H, NH), 10.96 (s, 1 H, NH); exact mass calcd for $C_{18}H_{26}N_2O_2$ 302.1995, found m/e 302.1991 (M^+).

***cis*-1,2,3,19,21,24-Hexahydro-2,3,7,8,12,13,17,18-octaethyl-1,19-dioxobilin (11).** The *cis*-3,4-dihydro-5′-formylpyrromethenone **9** (6.8 mg, 22 μmol) and the 5′(1*H*)-pyrromethenone **7** (6.1 mg, 22 μmol) were dissolved in 1.0 mL of CH_2Cl_2, 2,6-di-*tert*-butyl-4-methylpyridine[39] (4.5 mg, 22 μmol) and $POBr_3$[40] (2.0 mg, 7.3 μmol) were added, and the reaction was left for 1 at room temperature. The reaction then was diluted with 5 mL of CH_2Cl_2, washed successively with 2.5 mL each of saturated aqueous $NaHCO_3$, 1 M aqueous H_3PO_4, H_2O, and saturated aqueous NaCl, dried, and evaporated. The residue was purified by HPLC to give 5.7 mg (46%) of a blue solid: R_t (solvent B, 2.6 mL/min) s, min; UV/vis λ_{max} (log ϵ) 580 (4.01), 358 (4.50), 270 nm (4.26); NMR ($CDCl_3$) δ 1.00 (t, 3 H, CH_2CH_3, J = 7.5 Hz), 1.05–1.3 (m, 21 H, 7 × CH_2CH_3), 1.4–1.8 (m, 4 H, 2 × $CHCH_2CH_3$), 2.3–2.7 (m, 13 H, 6 × CH_2CH_3, and 4-H), 3.05–3.15 (m, 1 H, 3 H), 5.50, 6.01, 6.60 (3 × s, 3 H, 3 × meso-H); NMR (pyridine-d_5) 1.0–1.4 (m, 24 H, 8 × CH_2CH_3), 1.6–2.0 (m, 4 H, 2 × $CHCH_2CH_3$), 2.3–2.8 (m, 13 H, 6 × CH_2CH_3, and 4-H), 3.05–3.10 (m, 1 H, 3-H), 5.50, 6.23, 6.83 (3 × s, 3 H, 3 × meso-H); exact mass calcd for $C_{35}H_{48}N_4O_2$ 556.3777, found m/e 556.3769 (M^+).

***trans*-1,2,3,19,21,24-Hexahydro-2,3,7,8,12,13,17,18-octaethyl-1,19-dioxobilin (12).** The *trans*-3,4-dihydro-5′-formylpyrromethenone **10** was coupled and purified as described for **11**. The product was a blue solid (37%): R_t (solvent B, 2.6 mL/min) 18 min; UV/vis λ_{max} (log ϵ) 584 (4.00), 354 (4.54), 274 nm (4.30); NMR ($CDCl_3$) δ 0.95, 1.04 (2 × t, 6 H, 2 × CH_2CH_3, 2 × J = 7.5 Hz), 1.1–1.3 (m, 18 H, 6 × CH_2CH_3), 1.5–1.9 (m, 4 H, 2 × $CHCH_2CH_3$), 2.2–2.7 (m, 13 H, 6 × CH_2CH_3, and 4-H), 2.80 (m, 1 H, 3-H), 5.47 (d, 1 H, meso-H, J = 1.2 Hz), 6.00, 6.58 (2 × s, 2 H, meso-H); NMR (pyridine-d_5) δ 0.8–1.5 (m, 24 H, 8 × CH_2CH_3), 1.6–2.0 (m, 4 H, 2 × $CHCH_2CH_3$), 2.3–2.7 (m, 13 H, 6 × CH_2CH_3, and 4-H), 2.75–2.83 (m, 1H, 3-H), 5.50, 6.23, 6.83 (3 × s, 3 H, meso-H); exact mass calcd for $C_{35}H_{48}N_4O_2$ 556.3777, found m/E 556.3795 (M^+).

4-Ethyl-3-methyl-3-pyrrolin-2-one (13a).[27] The cyanohydrin of ethyl 2-methyl-3-oxopentanoate[41] was prepared through the bisulfite adduct[28] without purification and carried on directly to the pyrrolinone **13a**: mp 79–81 °C (lit.[27] mp 81–83 °C); NMR ($CDCl_3$) δ 1.10 (t, 3 H, 4-CH_2CH_3, J = 7.5 Hz), 1.77 (s, 3 H, 3-CH_3), 2.38 (q, 2 H, 4-CH_2CH_3, J = 7.5 Hz), 3.82 (s, 2 H, 5-CH_2), 7.68 (br s, 1 H, NH).

3-Ethyl-4-methyl-3-pyrrolin-2-one (13b).[23] Ethyl 2-ethyl-3-oxobutanoate[42] was converted as above to pyrrolinone **13b**:[26] mp 101–102 °C (lit.[23] mp 101 °C); NMR ($CDCl_3$) δ 1.06 (t, 3 H, 3-CH_2CH_3, J = 7.5 Hz), 1.97 (s, 3 H, 4-CH_3), 2.25 (q, 2 H, 3-CH_2CH_3, J = 7.5 Hz), 3.80 (s, 2 H, 5-CH_2), 8.14 (br s, 1 H, NH).

***tert*-Butyl 3′,4-Dimethyl-3-ethyl-4′-(2-(methoxycarbonyl)ethyl)-5-(1*H*)-pyrromethenone-5′-carboxylate (15a).** To a solution of 0.692 g (2.36 mmol) of *tert*-butyl 5-formyl-3-(2-(methoxycarbonyl)ethyl)-4-methylpyrrole-2-carboxylate (**14**)[30] and 0.433 g (3.47 mmol) of 4-ethyl-3-methylpyrrolin-2-one (**13a**) in 1.3 mL of MeOH was added 1.71 g (30.5 mmol) of KOH in 6.5 mL of H_2O at room temperature. The reaction was stirred for 18 h and then diluted with 26 mL of H_2O, and SO_2 (g) was passed through until the pH registered 3. The precipitate formed during the acidification was filtered, washed with 10 mM aqueous TFA, and dried to give 0.870 g (95%) of the 4′-propionic acid–pyrromethenone derivative. This acid was directly esterified by adding 1.2 mL of MeOH and then 23.6 mL of 0.1 M CH_2N_2 in ether[43] and stirring for 1 h at room temperature. The hetereogeneous reaction was filtered and the solid recrystallized from MeOH/ether/hexane to give 3.10 g (86%) of a yellow solid. The filtrate was evaporated and the residue was recrystallized from the same solvent to given an additional 0.26 g of product for a total yield of **15a** of 93%: mp 188–190 °C dec; UV/vis λ_{max} (log

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ε) 400 (sh), 380 (4.38), 257 (4.32), 250 nm (sh); NMR ($CDCl_3$) δ 1.18 (t, 3 H, 3-CH_2CH_3, J = 7.5 Hz), 1.56 (s, 9 H, $C(CH_3)_3$), 1.97 (s, 3 H, 4-CH_3), 2.10 (s, 3 H, 3′-CH_3), 2.45–2.65 (m, 4 H, 3-CH_2CH_3 overlapping 4′-$CH_2CH_2CO_2CH_3$), 3.01 (t, 2 H, 4′-$CH_2CH_2CO_2CH_3$, J = 7.5 Hz), 3.68 (s, 3 H, OCH_3), 5.98 (s, 1 H, meso-H); exact mass calcd for $C_{22}H_{30}N_2O_5$ 402.2155, found m/e 402.2158 (M^+).

***tert*-Butyl 3,3′-Dimethyl-4-ethyl-4′-(2-(methoxycarbonyl)ethyl)-5-(1*H*)-pyrromethenone-5′-carboxylate (15b).**[30] This compound was prepared as described.[30] The filtrate was evaporated and the residue was recrystallized from MeOH/ether/hexane to give **15b** in a total yield of 92%: mp 205–207 °C (lit.[30] mp 206–208 °C); exact mass calcd for $C_{22}H_{30}N_2O_5$ 402.2155, found m/e 402.2141 (M^+).

3′,4-Dimethyl-3-ethyl-4′-(2-(methoxycarbonyl)ethyl)-5(1*H*)-pyrromethenone (17a).[44] The 5′(*tert*-butyloxy)carbonyl)pyrromethenone **15a** (0.50 g, 1.2 mmol) was added to 20 mL of anhydrous trifluoroacetic acid and was stirred for 2 h at room temperature. The solution was poured into 200 mL of water and filtered, and the precipitate was washed with water and dried to give 0.37 g (98%) of **17a** as a greenish solid: mp 172–174 °C (lit.[44] mp 175–176 °C); UV/vis λ_{max} (log ε) 395 (5.32), 261 (4.83), 231 nm (4.77); NMR ($CDCl_3$) δ 1.18 (t, 3 H, 3-CH_2CH_3, J = 7.6 Hz), 1.94 (s, 3 H, 4-CH_3), 2.16 (s, 3 H, 3′-CH_3), 2.5–2.65 (m, 4 H, 3-CH_2CH_3 overlapping 4′-$CH_2CH_2CO_2CH_3$), 2.77 (t, 2 H, 4′-$CH_2CH_2CO_2CH_3$, J = 7.5 Hz), 3.69 (s, 3 H, OCH_3), 6.33 (s, 1 H, meso-H), 6.89 (d, 1 H, 5′-H, J = 2.7 Hz), 10.05 (br s, 1 H, NH), 11.63 (br s, 1 H, NH).

3,3′-Dimethyl-4-ethyl-4′-(2-(methoxycarbonyl)ethyl)-5(1*H*)-pyrromethenone (17b).[44] The 5′-(*tert*-butyloxy)carbonyl)pyrromethenone **15b** was converted to the 5′-H compound as described above in 99% yield: mp 200–202 °C (lit.[44] mp 203.5–205 °C); UV/vis λ_{max} (log ε) 394 (5.30), 259 (4.80), 230 nm (4.74); NMR ($CDCl_3$) δ 1.15 (t, 3 H, 4-CH_2CH_3, J = 7.5 Hz), 2.13 (s, 6 H, 3-CH_3, and 3′-CH_3), 2.41 (q, 2 H, 4-CH_2CH_3, J = 7.5 Hz), 2.60 (t, 2 H, 4′-$CH_2CH_2CO_2CH_3$, J = 7.5 Hz), 2.79 (t, 2 H, 4′-$CH_2CH_2CO_2CH_3$, J = 7.5 Hz), 3.70 (s, 3 H, OCH_3), 6.13 (s, 1 H, meso-H), 6.78 (d, 1 H, 5′H, J = 2.8 Hz).

3′,4-Dimethyl-3-ethyl-5′-formyl-4′-(2-(methoxycarbonyl)ethyl)-5-(1*H*)-pyrromethenone (16a).[45,46] The 5′-*tert*-butyloxycarbonylpyrromethenone **15a** (0.465 g, 1.16 mmol) was added to 2.3 mL of anhydrous trifluoroacetic acid and stirred for 2.5 h at room temperature. Trimethyl orthoformate (0.70 mL, 6.4 mmol) was added all at once, and after 5 min of stirring, the reaction was quenched by pouring it into 23 mL of water. The aqueous phase was extracted with 25% (v/v) isopropyl alcohol in chloroform (6 × 10 mL) and the combined organic phases were washed with 20 mL of water and 20 mL of saturated aqueous sodium chloride and dried. Evaporation and recrystallization of the residue from methanol/hexane gave 0.310 g (81%) of **16a** as a yellow solid: mp 207–209 (lit.[45] mp 205.5 °C; lit.[46] mp 211 °C); NMR ($CDCl_3$) δ 1.20 (t, 3 H, 3-CH_2CH_3, J = 7.6 Hz), 2.00 (s, 3 H, 4-CH_3), 2.14 (s, 3 H, 3′-CH_3), 2.55 (q, 2 H, 3-CH_2CH_3, J = 7.6 Hz), 2.61 (t, 2 H, 4′-$CH_2CH_2CO_2CH_3$, J = 7.7 Hz), 3.09 (t, 2 H, 4′-$CH_2CH_2CO_2CH_3$, J = 7.7 Hz), 3.68 (s, 3 H, OCH_3), 5.99 (s, 1 H, meso-H), 9.77 (s, 1 H, CHO), 10.69 (br s, 1 H, NH), 10.90 (br s, 1 H, NH); exact mass calcd for $C_{18}H_{22}N_2O_4$ 330.1580, found m/e 330.1583 (M^+).

3,3′-Dimethyl-4-ethyl-5′-formyl-4′-(2-(methoxycarbonyl)ethyl)-5-(1*H*)-pyrromethenone (16b).[9,45,46] The above procedure was used on the 5′-((*tert*-butyloxy)carbonyl)pyrromethenone **15b** to give a 76% yield of **16b** as a yellow solid: mp 201–203 °C (lit.[9] mp 202 °C; lit.[45] mp 218–220 °C; lit.[46] mp 212 °C); NMR ($CDCl_3$) δ 1.12 (t, 3 H, 4-CH_2CH_3, J = 7 Hz), 2.13 (s, 6 H, 3-CH_3 and 3′-CH_3), 2.45 (q, 2 H, 4-CH_2CH_3, J = 7.5 Hz), 2.60 (t, 2 H, 4′-$CH_2CH_2CO_2CH_3$, J = 7.6 Hz), 3.08 (t, 2 H, 4′-$CH_2CH_2CO_2CH_3$, J = 7.6 Hz), 3.67 (s, 3 H, OCH_3), 5.96 (s, 1 H, meso-H), 9.74 (s, 1 H, CHO), 10.58 (br s, 1 H, NH), 10.78 (br s 1 H, NH); exact mass calcd for $C_{18}H_{22}N_2O_4$ 330.1580, found m/e 330.1576 (M^+).

***cis*-3,4-Dihydro-3′,4-dimethyl-3-ethyl-5′-formyl-4′-(2-(methoxycarbonyl)ethyl)-5(1*H*)-pyrromethenone (18a).**[25,26] The 5′-formylpyrromethenone **16a** was reduced as described for the tetraethylpyrromethenone **8** above. The product was purified by HPLC to give **18a** as a slightly yellow solid in 37% yield: R_t (solvent C, 2.0 mL/min) 34.8 min (the corresponding pyrromethanone and dihydropyrromethanone were not eluted under these conditions); UV/vis λ_{max} (log ε) 350 (4.11), 240 nm (4.10). The NMR spectral assignments in $CDCl_3$ are found in Table I and the 3-H/4-H coupling constant is found in Table IV; exact mass calcd for $C_{18}H_{24}N_2O_4$ 332.1736, found m/e 332.1731 (M^+).

***cis*-3,4-Dihydro-3,3′-Dimethyl-4-ethyl-5′-formyl-4′-(2-(methoxycarbonyl)ethyl)-5(1*H*)-pyrromethenone (18b).**[25,26] The above procedure was carried out on pyrromethenone **16b** to give a 44% yield of **18b** after preparative HPLC purification: R_t (solvent C, 2.0 mL/min) 34.8 min (the corresponding pyrromethanone and dihydropyrromethanone were not eluted under these conditions); UV/vis λ_{max} (log ε) 354 (4.12), 241 nm (4.11). The NMR spectral assignments are found in Table I and the 3-H/4-H coupling constant is found in Table IV; exact mass calcd for $C_{18}H_{24}N_2O_4$ 332.1736, found m/e 332.1738 (M^+).

***trans*-3,4-Dihydro-3′,4-dimethyl-3-ethyl-5′-formyl-4′-(2-(methoxycarbonyl)ethyl)-5-(1*H*)-pyrromethenone (19a).**[25,26] The corresponding *cis*-dihdyropyrromethenone **18a** (0.100 g, 0.30 mmol) was heated under reflux in 30 mL of 0.5 M sodium methoxide in methanol for 2.5 h and then cooled, 15 mL of 1.0 M aqueous KH_2PO_4 was added, and the methanol was evaporated. The residue was extracted with 25% (v/v) isopropyl alcohol in methanol (6 × 15 mL), and the combined organic phase was washed with 15 mL of water and 15 mL of saturated aqueous NaCl and dried. Evaporation left a residue which was purified by preparative HPLC to give **19a** as a slightly yellow solid in 48% yield (48 mg): R_t (solvent C, 2.0 mL/min) 31.3 min (the corresponding pyrromethanone was not eluted under these conditions); UV/vis λ_{max} (log ε) 351 (4.11), 242 nm (4.09). The NMR spectral assignments in $CDCl_3$ are found in Table I and the 3-H/4-H coupling constant is found in Table IV; exact mass calcd for $C_{18}H_{24}N_2O_4$ 332.1736, found m/E 332.1727 (M^+).

***trans*-3,4-Dihydro-3,3′-dimethyl-4-ethyl-5′-formyl-4′-(2-(methoxycarbonyl)ethyl)-5(1*H*)-pyrromethenone (19b).**[25,26] The procedure as described for **19a** above was used to give a 45% yield of **19b**: R_t (solvent C, 2.0 mL/min), 31.3 min (the corresponding pyrromethanone was not eluted under these conditions); UV/vis λ_{max} (log ε) 353 (4.13), 241 nm (4.10). The NMR spectral assignments in $CDCl_3$ are found in Table I and the 3-H/4-H coupling constant is found in Table IV; exact mas calcd for $C_{18}H_{24}N_2O_4$ 332.1736, found m/e 332.1726 (M^+).

***cis*-3,18-Diethyl-1,2,3,19,21,24-hexahydro-2,7,13,17-tetramethyl-1,19-dioxobilin-8,12-dipropionic Acid Methyl Ester (20a).**[25] This compound was prepared by coupling **18 and 17b** as described for dioxobilin **11**. The product was purified by preparative HPLC to give 41% of **20a** as a blue solid: R_t (solvent D, 2.0 mL/min) 30 min. NMR spectral properties are found in Tables II, III, and IV; exact mass calcd for $C_{35}H_{44}N_4O_6$ 616.3261, found m/e 616.3270 (M^+).

***cis*-2,17-Diethyl-1,2,3,19,21,24-hexahydro-3,7,13,18-tetramethyl-1,19-dioxobilin-8,12-dipropionic Acid Methyl Ester (20b).**[25] This compound was prepared by coupling **18b** and **17a** as described for dioxobilin **11**. The product was purified by preparative HPLC to give 45% of **20b** as a blue solid: R_t (solvent D, 2.0 mL/min) 32 min. NMR spectral properties are found in Tables II, III, and IV; exact mass calcd for $C_{35}H_{44}N_4O_6$ 616.3261, found m/e 616.3236 (M^+).

***trans*-3,18-Diethyl-1,2,3,19,21,24-hexahydro-2,7,13,17-tetramethyl-1,19-dioxobilin-8,12-dipropionic Acid Methyl Ester (21a).**[26] This compound was prepared by coupling **19a** with **17b** as described for dioxobilin **11**. The product was purified by preparative HPLC to give 58% of **21a** as a blue solid: R_t (solvent D, 2.0 mL/min) 25 min; UV/vis λ_{max} (log ε) 583 (4.06), 343 (4.45), 272 nm (4.25). NMR spectral properties are found in Tables II, III, and IV; exact mass calcd fro $C_{35}H_{44}N_4O_6$ 616.3261, found m/e 616.3233 (M^+).

***trans*-2,17-Diethyl-1,2,3,19,21,24-hexahydro-3,7,13,18-tetramethyl-1,19-dioxobilin-8,12-dipropionic Acid Methyl Ester (21b).**[25,26] This compound was prepared by coupling **19b** with **17a** as described for dioxobilin **11**. The product was purified by preparative HPLC to give 36% of **21b** as a blue solid: R_t (solvent D, 2.0 mL/min) 25 min. NMR spectral properties are found in Tables II, III, and IV; exact mass calcd for $C_{35}H_{44}N_4O_6$ 616.3261, found m/e 616.3247 (M^+).

3,4-Diethyl-1*H*-pyrrole-2,5-dione (2,3-Diethylmaleimide).[47,48] 3,4-Diethylpyrrole[20,21,49] (10 g, 81 mmol) in 15 mL of pyridine containing 12 mL of 30% aqueous H_2O_2 was heated at reflux for 5 min. Another 3 mL of H_2O_2 was added and heating continued for 10 more min.[22] The solvent was evaporated and the residue was taken up in 100 mL of $CHCl_3$. The organic phase was washed with 30 mL of 1 M NaOH, dried, and cautiously evaporated, leaving the crude pyrrolinone (10.0 g, 97%). This crude product was dissolved in 175 mL of acetone, a solution of 17.1 g (171 mmol) of CrO_3 in 170 mL of 2 M H_2SO_4 was added, and the reaction was heated at 50 °C for 1 h. After the solution was cooled to room temperature, the acetone was evaporated and the aqueous residue was extracted with EtOAc (7 × 100 mL). The combined organic phase was washed with 100 mL of saturated aqueous NaCl, dried, and evaporated, and the residue was purified by silica gel chromatography using

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isooctane/ether, 1/1, to give 5.8 g (47%) of 2,3-diethylmaleimide: mp 69-70 °C (lit.[47] mp 68 °C, lit.[48] mp 68-70 °C); R_t (GC) 3.30 min.

***cis*-3,4-Diethylpyrrolidine-2,5-dione (*cis*-2,3-Diethylsuccinimide).** To 180 mg (0.85 mmol) of the above 3,4-diethyl-1*H*-pyrrole-2,5-dione in 10 mL of ethyl acetate was added 18 mg of PtO_2. Hydrogen at 50 psi was applied for 6 h,[31] the solution was filtered through Celite, and the filtrate was evaporated to give 132 mg (100%) of residue. GC analysis showed a 95/5 mixture of cis/trans isomers which may be due to epimerization during analysis, as the cis compound was never found completely free of the trans by GC analysis.[50] The product was recrystallized from MeOH/H_2O and sublimed at 70 °C/1 torr to give a 98/2 ratio of cis/trans 2,3-diethylsuccinimide as white crystals: mp 87-89 °C; R_t (GC) cis 5.16 min, trans 4.17 min; UV λ_{max} (log ϵ) 246 (1.93), 221 nm (2.28); IR (KBr) 3130, 1670, 1340 cm^{-1}; NMR ($CDCl_3$, 90 MHz) δ 1.02 (t, 6 H, 2 × CH_2CH_3, J = 7.5 Hz), 1.54-1.77 (m, 4 H, 2 × CH_2CH_3), 2.70-2.83 (m, 2 H, 2 × CH), 8.32 (br, s, 1 H, NH). Anal. Calcd for $C_8H_{13}NO_2$: C, 61.9; H, 8.4; N, 9.0. Found: C, 61.9; H, 8.4; N, 8.8.

***trans*-3,4-Diethylpyrrolidine-2,5-dione (*trans*-2,3-Diethylsuccinimide).** To the above *cis*-3,4-diethylpyrrolidine-2,5-dione (153 mg, 1.00 mmol) was added a solution of 2.24 g (2.00 mmol) of potassium *tert*-butoxide in 60 mL of *tert*-butyl alcohol. After refluxed for 2 h, the solution was cooled and the *tert*-butyl alcohol was evaporated. Aqueous 1 M H_3PO_4 (20 mL) was added and the aqueous phase was extracted with CH_2Cl_2 (5 × 10 mL) which was dried and evaporated to give 143 mg (93%) of *trans*-2,3-diethylsuccinimide, mp 59-60 °C after recrystallization from H_2O and sublimation at 70 °C/1 torr: R_t (GC) 4.17 min; UV λ_{max} (log ϵ) 244 (1.64), 220 nm (2.22); IR (KBr) 3140, 1670, 1175 cm^{-1}; NMR ($CDCl_3$, 90 MHz) δ 1.00 (t, 6 H, 2 × CH_2CH_3, J = 7.5 Hz), 1.60-1.90 (m, 4 H, 2 × CH_2CH_3), 2.42-2.53 (m, 2 H, 2 × CH), 8.80 (br, s, 1 H, NH). Anal. Calcd for $C_8H_{13}NO_2$: C, 61.9; H, 8.4; N, 9.0. Found: C, 62.2; H, 8.4; N, 8.9.

3-Ethyl-4-methyl-1*H*-pyrrole-2,5-dione (2-Ethyl-3-methylmaleimide).[15,47,51] This compound was prepared from 3-ethyl-4-methylpyrrole[20,21,52] as described for the 3,4-diethyl-1*H*-pyrrole-2,5-dione above in 45% yield: mp 66-68 °C (lit.[15] mp 66-67 °C; lit.[47] mp 67-68 °C; lit.[51] mp 68 °C); R_f (TLC) 0.89.

***cis*-3-Ethyl-4-methylpyrrolidine-2,5-dione (*cis*-2-Ethyl-3-methylsuccinimide).**[15,31] This compound was prepared as described[15] in quantitative yield: mp 48-50 °C (lit.[31] mp 50 °C); R_f (TLC) cis 0.20, trans 0.30; R_t (GC) cis 1.9 min, trans 1.3 min.[50]

***trans*-3-Ethyl-4-methylpyrrolidine-2,5-dione (*trans*-2-Ethyl-3-methylsuccinimide).**[31] This compound was prepared in an analogous manner to the *trans*-3,4-diethylpyrrolidine-2,5-dione above in 64% yield: mp 57-59 °C (lit.[31] mp 60-61 °C); R_f (TLC) 0.30; R_t (GC) 1.3 min.

Bile Pigment Oxidative Degradations.[32] To a solution of 250 mg of $Na_2Cr_2O_7$ in 6 mL of H_2O was added 10 mg of the bile pigment in 2 mL of THF. The oxidation was allowed to proceed at room temperature for 2 h, then 10 mL of H_2O was added, and the mixture was extracted with CH_2Cl_2 (5 × 10 mL). The combined organic phase was dried and evaporated. The residue of oxidation products from the octaethyldioxotetrapyrrole was analyzed by GC. In addition to the succinimide and maleimide products, an incomplete degradation product appears with a R_t 8.30 min. The oxidation products from the bile pigments **20a**, **20b**, **21a**, and **21b** were analyzed by TLC.[33,34,37] In addition to the succinimide and 2-ethyl-3-methylmaleimide, 3-(2-(methoxycarbonyl)ethyl)-4-methyl-1*H*-pyrrole-2,5-dione (hematinic acid methyl ester) was seen at R_f 0.50.

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Catalytic Cleavage of Active Phosphate and Ester Substrates by Iodoso- and Iodoxybenzoates

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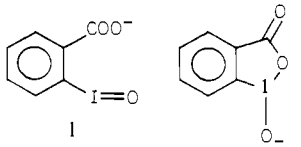
Abstract: *p*-Nitrophenyl acetate, *p*-nitrophenyl hexanoate, and *p*-nitrophenyl diphenyl phosphate (PNPDPP) were cleaved by *o*-iodosobenzoate, *o*-iodoxybenzoate, and 5-(*n*-octyloxy)-2-iodosobenzoate (**3**) in aqueous micellar cetyltrimethylammonium chloride solutions at pH 8. The system **3**/CTACl was the best catalyst and PNPDPP was the most reactive substrate. In a remarkably rapid hydrolytic reaction at 25 °C, 1.0×10^{-5} M PNPDPP was cleaved by 7.14×10^{-5} M **3** in 2.0×10^{-4} M CTACl with k_ψ = 1.04 s^{-1}. Experiments in which [PNPDPP] > [**3**] demonstrated that the catalyst "turned over"; i.e., degradation of an intermediate phosphate was not rate limiting.

When solubilized in pH 8 micellar cetyltrimethylammonium chloride (CTACl), *o*-iodosobenzoate (**1**) is an efficient catalyst



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for the cleavage of *p*-nitrophenyl acetate (PNPA) and *p*-nitrophenyl diphenyl phosphate (PNPDPP).[1] The actual source of catalytic power is 1-hydroxy-1,2-benziodoxolin-3-one (1,3-dihydro-1-hydroxy-3-oxo-1,2-benziodoxole) (**2**) the valence tautomeric form in which **1** prefers to exist[2] and which appears to be a powerful O nucleophile. In this full report, these results are

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