Synthesis of Bilin-1,19-diones and Biladiene-*ac*-1,19-diones with C(10) Adamantyl and *tert*-Butyl Groups

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Bilirubin and biliverdin analogs bearing bulky adamantyl and *tert*-butyl groups at the central C(10) position were synthesized and their structures were analyzed by nmr and ultraviolet-visible spectroscopy and by molecular mechanics calculations, all of which collectively indicated a preference for helical conformations.

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Introduction.

Biliverdin is a blue-green pigment formed in plant and animal metabolism by oxidative cleavage of the heme porphyrin macrocycle (Figure 1) [1-3]. In mammals, biliverdin is reduced rapidly and efficiently to bilirubin, the yellow pigment of jaundice, which is eliminated in normal metabolism following esterification in the liver by a glucuronosyl transferase and excretion into bile [1,4]. In other animals, biliverdin is excreted directly [1]. Bilirubin, which is cytotoxic [5] and a powerful antioxidant [6], is produced in a healthy human adult at a rate of ~300 mg/day, principally from the breakdown of red blood cells. Although it is intrinsically unexcretable, bilirubin is efficiently eliminated by the liver following uptake and enzymic conversion to water-soluble glucuronides that are promptly secreted into bile. Impaired excretion of the glucuronides occurs in many types of hepatobiliary disease, but retention of native bilirubin is principally observed in newborn babies [1,4,7]. Accumulation of either native bilirubin or its glucuronides in the body is manifested in jaundice.

Bilirubin belongs to the class of pigments called "linear tetrapyrroles"; yet, its solution and biological properties do not correlate well with either a linear or the porphyrin-like (Figure 1) shape in which the polar carboxyl and lactam groups are freely solvated [1,3,7]. Its preferred shape is now known to be neither linear nor porphyrin-like, which are sterically disfavored conformations [8]. Rather, bilirubin is bent in the middle and adopts the shape of a half-opened book or a ridge-tile (Figure 1, inset). In this conformation the propionic acid groups readily engage in intramolecular hydrogen bonding to the opposing dipyrrinone lactam and pyrrole components [8]. In bilirubin and its congeners with propionic acids at C(8) and C(12), this provides considerable conformational stabilization of the ridge-tile shape while tucking the polar groups inward and rendering the pigment surprisingly lipophilic [9]. Interestingly, even when the carboxylic acid groups are absent, bilirubin analogs preferentially adopt a ridge-tile shape, although the energy difference between it and others that are bent or folded in the middle is not nearly as great as in bilirubin [10].

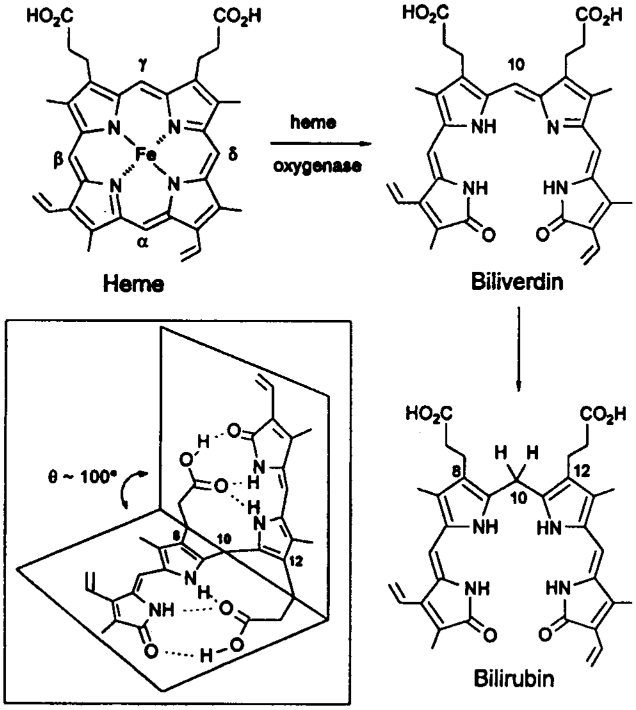
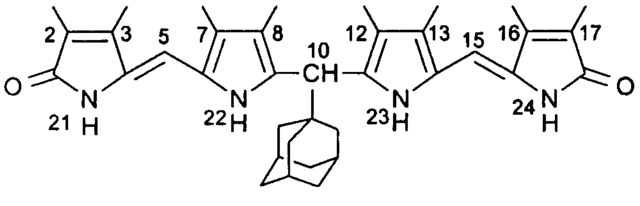


Figure 1. Conversion of heme to biliverdin and bilirubin. Biliverdin and bilirubin are shown in a porphyrin-like conformation, which is preferred in the former and disfavored in the latter. (Inset) Ridge-tile conformation of bilirubin stabilized by intramolecular hydrogen bonding (shown by dashed lines).

Falk *et al.* [11] recently showed that bulky hydrocarbon groups located at the central C(10) carbon of biliverdin have a powerful conformation-distorting and stabilizing effect on its structure. For example, a C(10)-*tert*-butyl group is thought to cause the verdin to twist from its favored helical porphyrin-like conformation (shaped like a lock washer) into a ridge-tile conformation. This change in conformation is immediately noticeable because the typical blue-green color of the verdin is shifted to reddish-yellow, signifying a large increase in the pitch of the helix. In confirmation of such a major distortion, nmr and semi-empirical PPP-MO calculations of the ultraviolet-visible spectrum predict that the interplanar angle of the two dipyrrinones is some 70°, which is closer to the ~98° interplanar angle found in the ridge-tile conformation of bilirubin [8,12] than the ~12° angle found in helical biliverdin dimethyl ester [2,13,14].

To explore whether bulky alkyl groups at C(10) of bilirubins might further stabilize the favored ridge-tile conformation, we synthesized bilirubin analogs with C(10) adamantyl and *tert*-butyl groups (**1** and **2**, respectively). For this study we chose rubins which do not have propionic acid groups at C(8) and C(12) and thus cannot stabilize the ridge-tile conformation through intramolecular hydrogen bonding. For comparison with earlier work on a C(10)-*tert*-butyl verdin [11], the corresponding C(10) adamantyl and *tert*-butylverdins (**3** and **4**, respectively) were also synthesized.



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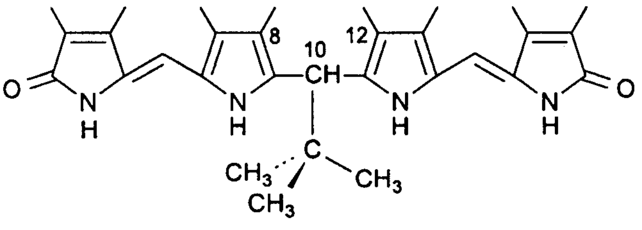

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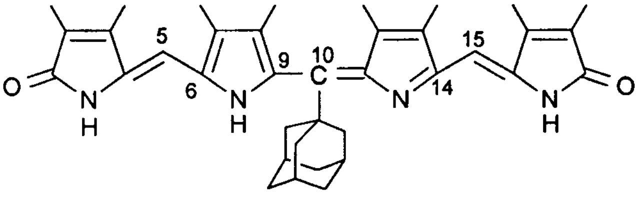

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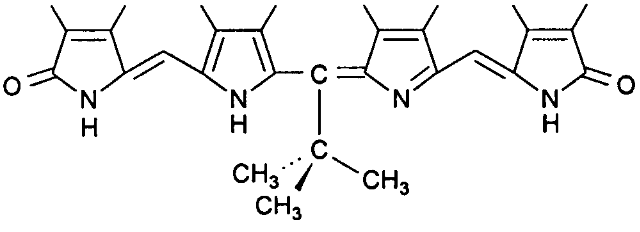

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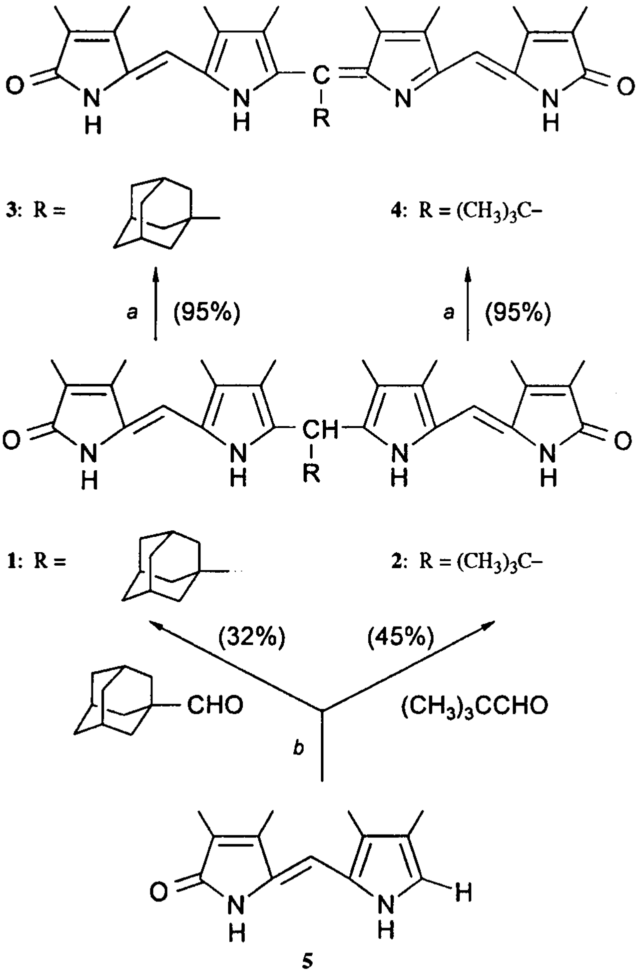
Synthesis.

Synthesis of the tetrapyrroles **1-4** followed logically from the known [15] dipyrrinone **5** (3,4-dimethyl-5-(3,4-dimethyl-2-ylmethylidene)-3-pyrrolin-2-one) as outlined in the Synthetic Scheme. To prepare **1** and **2**, we modified Falk's condensation procedure [11], substituting trifluoroacetic acid for *p*-toluenesulfonic acid and shortening the time for the reaction run at room temperature in dichloromethane under nitrogen. Reaction of **5** with 1-adamantyl aldehyde [16] gave the bright yellow rubin **1** following flash chromatography on silica gel and crystallization from dichloromethane methanol. Similarly, **5** was reacted with pivaldehyde to afford rubin **2**. Rubins **1** and **2** could be converted to their respective verdins, **3** and **4**, by oxidation using 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in tetrahydrofuran at room temperature during thirty minutes. Consistent with Falk's observation [11] the purified verdins, in defiance of the name "verdin," were bright red in the solid or in solution.

Synthetic Scheme



a tetrahydrofuran, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; *b* dichloromethane, trifluoroacetic acid.

The 1-formyladamantane used in the preparation of **1** was prepared by Swern oxidation [16] of 1-hydroxymethyladamantane, which was synthesized in three steps from adamantane [17,18]. The Swern oxidation gave pure aldehyde in 78% yield, an improvement over other methods.

Spectroscopic Properties.

The carbon-13 nuclear magnetic resonance (^{13}C-nmr) chemical shifts of the rubin backbone of **1** and **2** are very similar (Table 1), consistent with the prevalence of comparable conformations in dimethyl sulfoxide solvent. The corresponding verdins, **3** and **4**, also exhibit rather similar ^{13}C-nmr chemical shifts, with a doubling of signals, for the verdin backbone, again consistent with similar conformations in deuterated chloroform solvent.

Table 1

^{13}C-NMR Chemical Shifts and Assignments [a] for Rubins **1** (R = 1-adamantyl) and **2** (R = *tert*-butyl), and for Their Corresponding Verdins **3** and **4**, Respectively

	Chemical Shift in dimethyl-d_6 sulfoxide		Chemical Shift in chloroform-d [c]	
Carbon [b]	**1**	**2**	**3**	**4**
1,19 C=O	172.2	172.4	173.1	173.3
			172.1	172.3
2,18 C=	123.1	123.1	123.4	123.6
			147.0	146.8
2^1,18^1 CH_3	8.6	8.7	10.0	10.0
			9.8	9.6
3,17 C-	141.5	141.7	141.8	142.0
			140.9	140.9
3^1,17^1 CH_3	9.8	10.0	10.2	10.2
			10.1	10.1
4,16 C=	130.4	131.5	130.1	130.2
			130.7	130.7
5,15 CH=	99.1	99.4	95.6	98.3
			98.2	98.7
6,14 C=	125.3	125.4	124.7	124.8
			166.8	167.2
7,13 C=	120.6	120.7	124.0	124.1
			151.0	150.8
7^1,13^1 CH_3	10.4	10.5	8.8	8.8
			8.9	9.0
8,12 C=	117.5	117.2	156.6	156.7
			134.2	134.3
8^1,12^1 CH_3	10.0	10.1	11.2	11.3
			10.5	10.4
9,11 C=	132.2	132.3	131.2	131.8
			142.6	141.8
10 CH or C=	45.6	44.3	117.8	117.8
10^1 C	38.4	36.7	42.4	23.4
10^2 CH_2 or CH_3	41.2	29.4	43.0	32.3
10^3 CH	28.5	-	29.4	-
10^4 CH_2	36.8	-	37.1	-

[a] Run at 2.5×10^{-2} *M* concentration of pigment at 22°. Multiplicities are determined by the attached proton test method; [b] Superscripts refer to carbons in the β-substituent chains, *e.g.* 2^1 is the first carbon attached to ring carbon 2; [c] Run in chloroform-d containing 40% dimethyl-d_6 sulfoxide. Assignments were made by attached proton test, nOe, HMQC and HMBC experiments.

Consistent with the ^{13}C-nmr results in dimethyl sulfoxide-d_6 solvent, the relevant ^1H-nmr chemical shifts of **1** and **2** show great similarity (Table 2). In deuterated chloroform, they differ only slightly: in the chemical shifts of the methyls at carbons 8(12) and 2(18) and the pyrrole NH at 22(23). Whether these small changes indicate slightly different conformations for **1** and **2** in chloroform is unclear. Earlier studies on rubin esters indicate dimer formation in chloroform [2], and perhaps **1** and **2** adopt slightly different conformations to accommodate dimer formation.

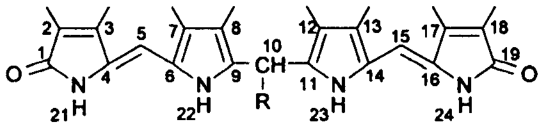
More significant differences could be detected in the ^1H-nmr spectra of verdins **3** and **4** in deuterated chloroform (Table 2). As in the ^{13}C-nmr, there is a doubling of verdin signals, but there are large differences between the NH chemical shifts of **3** and **4**. Whether this, too, reflects differing abilities in these verdins to form intermolecular hydrogen bonds between the dipyrrinones [2] or whether it reflects different conformations is unclear from the data.

Homonuclear Overhauser effects (nOes) support the structural conclusions reached above. Thus, in **1** and **2** a strong nOe between the pyrrole and lactam hydrogens (Figure 2) is observed, confirming a *syn-Z* configuration of the dipyrrinones. Consistent with this, nOes were found between the C(5)/C(15) hydrogens and the methyl groups at C(3) and C(7)/C(13) and C(17). NOes between the *tert*-butyl methyls or corresponding adamantyl methylenes and the pyrrole NHs were found. In **3** and **4** a similar set of nOes between the N(21) and N(22) lactam and pyrrole hydrogens confirmed the *syn-Z* dipyrrinone conformation, as did nOes between the C(15) hydrogen and methyls at C(13) and C(17). A bent conformation near C(10) is supported by nOes between the *tert*-butyl methyls or corresponding adamantyl methylenes and the pyrrole/lactam NHs.

Ultraviolet-visible spectroscopy has been used to detect non-linear behavior in Beer's Law (due to self-association) by varying the pigment concentration and temperature [2], and it has been used to extract information on the conformation of rubins [8]. The latter is based on the behavior of bichromophoric molecular excitons. Rubins such as **1** and **2** have two dipyrrinone chromophores each that interact through electric transition dipole-dipole (exciton) coupling [8,19,20] to give two electronic transitions (ultraviolet-visible absorption bands) flanking the isolated dipyrrinone transition. The relative orientation of the dipyrrinones (and the relevant dipyrrinone long wavelength electric dipole transition moments) determines the intensity (or allowedness) of the two exciton transitions and their separation (splitting). The ultraviolet-visible spectral data for **1** and **2** (Table 3) clearly indicate two exciton absorptions centered near 390 and 420 nm. The more intense shorter wavelength band signifies a helical

Table 2

^{1}H-NMR Chemical Shifts, Multiplicities and Assignments [a] for Rubins **1** (R = 1-adamantyl) and **2** (R = *tert*-butyl), and for Their Verdins **3** and **4**, Respectively



	Chemical Shifts in chloroform-d				in dimethyl-d_6 sulfoxide	
Proton [b]	**1**	**2**	**3**	**4**	**1**	**2**
21,24 NH	9.62 (brs)	9.64 (brs)	10.12 (brs) 9.62 (brs)	11.28 (brs) 10.50 (brs)	9.36 (brs)	9.38 (brs)
22,23 NH	8.70 (brs)	8.30 (brs)	9.40 (brs)	10.22 (brs)	9.70 (brs)	9.68 (brs)
5,15 CH=	5.85 (s)	5.85 (s)	5.45 (s) 5.35 (s)	5.82 (s) 6.13 (s)	5.99 (s)	5.97 (s)
10 CH	4.05 (s)	4.17 (s)	-	-	3.92 (s)	4.04 (s)
10^1 C	-	-	-	-	-	-
10^2 CH_2/CH_3	1.66 (m)	1.15 (s)	1.69 (m)	1.46 (s)	1.59 (m)	0.97 (s)
10^3 CH	2.02 (m)	-	1.22 (m)	-	1.93 (m)	-
10^4 CH_2	1.63 (m)	-	1.31 (m)	-	1.59 (m)	-
8^1,12^1 CH_3	1.70 (s)	1.58 (s)	1.38 (s) 1.35 (s)	1.84 (s) 1.35 (s)	1.76 (s)	1.73 (s)
7^1,13^1 CH_3	2.14 (s)	2.12 (s)	1.58 (s) 1.42 (s)	2.13 (s) 1.91 (s)	2.06 (s)	2.02 (s)
3^1,17^1 CH_3	2.04 (s)	2.12 (s)	1.58 (s) 1.58 (s)	2.02 (s) 2.10 (s)	1.95 (s)	1.92 (s)
2^1,18^1 CH_3	1.76 (s)	2.01 (s)	1.32 (s) 1.50 (s)	1.35 (s) 1.95 (s)	1.93 (s)	1.88 (s)

[a] Run at 4 x 10^{-3} *M* concentration of pigment at 22°; [b] Superscripts refer to carbons in the β-substituent chains, *e.g.* 2^1 is the first carbon attached to ring carbon 2.

rubin conformation as opposed to a stretched conformation [8]. Unlike rubin esters [2] and the parent rubin, there is very little change in the ultraviolet-visible spectra over a wide range of solvent polarity and type. This observation suggests conformational homogeneity over a range of solvent polarity. Should intermolecular hydrogen bonding occur in **1** and **2**, as it does in the parent in nonpolar solvents, it apparently does not influence the pigment conformation.

The ultraviolet-visible spectra of verdins **3** and **4** show that the typical verdin long wavelength absorption band near 650 nm has been shifted to near 500 nm (or near 540 nm in dimethyl sulfoxide), consistent with the observation that **3** and **4** are not blue-green but bright red. The most intense ultraviolet-visible band in a verdin spectrum lies near 390 nm. In **3** and **4** this is split into two bands of nearly equal intensity, one near 410 nm, the other near 390 nm, as predicted by the molecular orbital calculations of Falk *et al.* [11] for a verdin conformation with torsion angles 20°, 85°, 20° about the C(5)-C(6), C(9)-C(10) and C(14)-C(15) bonds.

Molecular Dynamics Calculations.

Consistent with the spectroscopic analyses above, molecular dynamics calculations [21] on rubins **1** and **2** indicate that the minimum energy conformations adopt a helical shape (Figure 3). The relevant structure parameters may be found in Table 4. The molecular shape-defining torsion angles about the C(10) methylene group are oppositely-signed: ~ -39° and ~ +74° for the C(9)-C(10) and C(10)-C(11) bonds, respectively, of **1**, where 0° is the angle in a porphyrin-like conformation of Figure 1. In **2**, the torsion angles are ~ -38° and ~ +71°, respectively; whereas, in the parent rubin they are ~ -44° and ~ +79°. The shape near the center of **1**, **2** and their parent rubin is somewhat like that found in an X-ray crystallographic structure of a dipyrrylmethane (5,5'-diethoxycarbonyl-3,3',4,4'-tetraethyldipyrrol-2-ylmethane) [22], where the torsion angles about the central CH_2 were also found to be oppositely-signed and even larger (+67°, -94°).

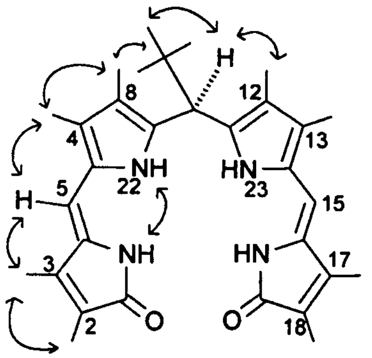
The interplanar angle (or dihedral angle) made by the two dipyrrinones (average plane) in **1** is ~31° and in **2**

Table 3

Solvent Dependence of the Ultraviolet-Visible Spectroscopic Data [$\varepsilon^{max}(\lambda^{max}$] [a] for 10-(1-Adamantyl)rubin (**1**), 10-*tert*-Butylrubin (**2**), 10-(1-Adamantyl)verdin (**3**), and 10-*tert*-Butylverdin (**4**)

Solvent	**1**	**2**	Parent Rubin	**3**	**4**
Benzene	46500 (382) 20800 (422) sh	55700 (382) 25300 (422) sh	32100 (387)	13900 (399) 15400 (415) sh 14600 (433) sh 5790 (496)	30100 (393) sh 36400 (415) 7070 (510)
Chloroform	42900 (390) sh 68600 (407)	46200 (390) sh 52700 (405)	44500 (387)	31000 (392) sh 34300 (412) 7850 (496)	29900 (386) sh 32900 (408) 7920 (493)
Dichloromethane	50100 (381) 48300 (401) sh	45700 (387) 43800 (400) sh	37700 (380)	26800 (396) sh 28600 (411) 6910 (484)	30000 (387) sh 31500 (405) 6290 (490)
Acetone	43500 (380) 26000 (409) sh	49700 (381) 27500 (408) sh	27700 (379) 17800 (410) sh	31000 (389) sh 33220 (407) 6520 (515)	38200 (383) sh 41800 (404) 8070 (505)
Methanol	41800 (396) 33300 (423) sh	37100 (395) 29700 (420) sh	33000 (395) sh 33700 (423)	34100 (388) sh 40800 (415) 6860 (512)	34000 (384) sh 40400 (411) 7150 (508)
Dimethyl sulfoxide	37800 (389) 26400 (421) sh	44300 (388) 33400 (408) sh	40300 (396) sh 46400 (426)	34500 (389) sh 39800 (416) 5490 (542)	38700 (389) sh 43800 (414) 7510 (543)

[a] Solutions were 10^{-5} *M* in pigment λ^{max} in nm, ε^{max} in L • mol^{-1} • cm^{-1}; sh = shoulder.



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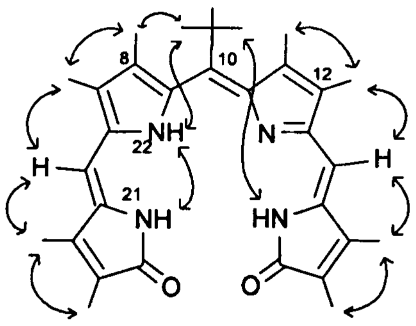

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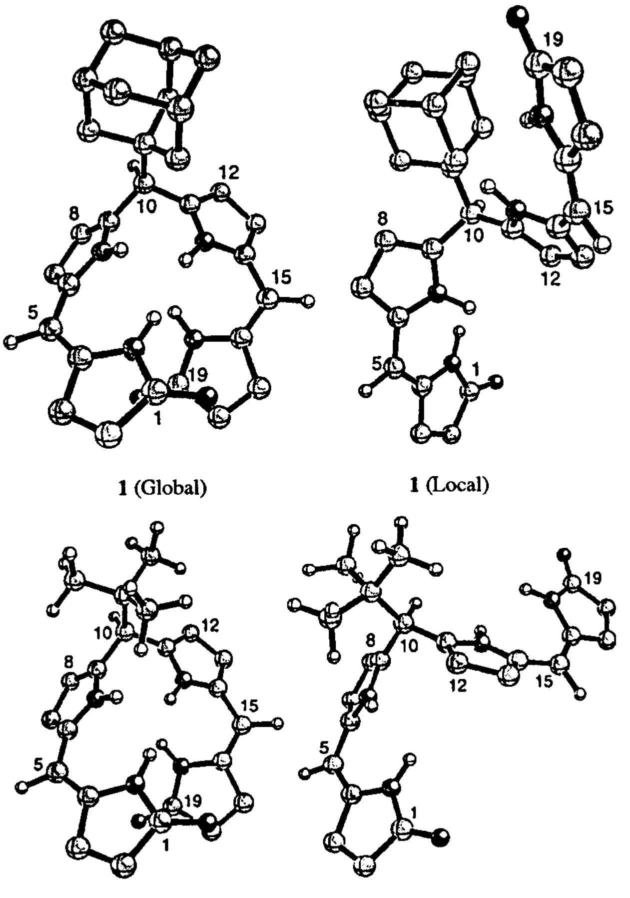
Figure 2. Homonuclear Overhauser effects recorded for **2** and **4** shown by double-headed arrows. Similar data were recorded for **1** and **3**.

~26°, which are much smaller than that of bilirubin (Figure 1, inset, θ ~100°). However, bilirubin has *planar* dipyrrinones as well as intramolecular hydrogen bonding, and the individual dipyrrinones of **1** and **2** are not planar but *twisted* slightly about the C(5)-C(6) and C(14)-C(15) single bonds: ~ +40° and ~ -32°, respectively. In the parent rubin, they are also twisted: ~ +41° and ~ -35°. Such twisting occurs largely from rotations about the carbon-carbon single bonds, as the carbon-carbon double bonds are only very slightly distorted out of planarity, C(4)=C(5): ~ +6° and C(15)=C(16): ~ -1° in **1**, **2** and the parent.

The large difference between the dihedral angle of bilirubin and those of **1**, **2** and the parent thus comes at least in part from twisting in the dipyrrinones. The choice of reference frame also dictates the magnitude of the dihedral angle. In the dipyrrylmethane reference frame, the dihedral angles defined by the dipyrrinone *pyrrole rings* (only) and C(10) of **1**, **2** and the parent are all larger and rather similar in magnitude: ~69°, ~67° and ~74°, respectively, while remaining oppositely-signed. In bilirubin, they remain unchanged because its dipyrrinones are planar. Thus, the influence of the C(10) adamantyl and *tert*-butyl groups appears to stabilize but not distort the lowest energy *helical conformation* of the parent rubin.



1 (Global) **1** (Local)

2 (Global) **2** (Local)

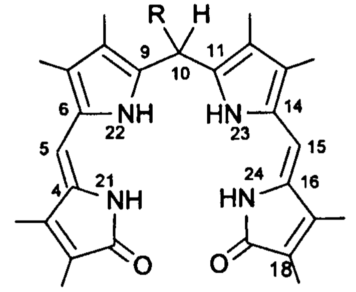
Figure 3. Ball and stick conformational representations for helical global (left) and local (right) energy minimum conformations of rubins **1** and **2**. Local minimum energy conformations lie 2-7 kcal/mole higher. Certain hydrogens and methyl groups are removed for clarity of representation.

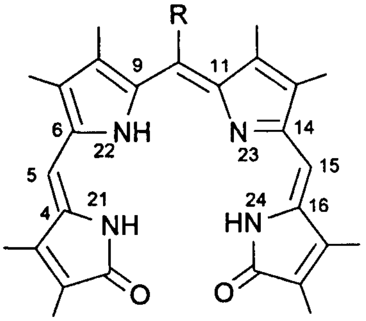
The nearest energy local minimum conformations of **1** and **2** lie some 5 kcal/mole above the global minimum. The conformations at these local minima are similar to each other but rather different from the global minima (Figure 3). Although the dipyrrinones are twisted, as in the global minima, the torsion angles about C(10) are not oppositely signed (Table 4). The net result is a local minimum conformation that is more like the ridge-tile conformation of bilirubin and less like the helical global minima.

Molecular dynamics calculations on verdins **3** and **4** also indicate a preference for helical structures (Figure 4). In the absence of the bulky C(10) substituent, the parent verdin is predicted to adopt a slightly helical conformation with very little distortion near C(10). The torsion angles about C(4)=C(5), C(5)-C(6), C(9)-C(10), C(10)=C(11), C(14)-C(15) and C(15)=C(16) are computed to be 3.3, 26, 5, 2, 26 and 3.9 degrees, respectively, as compared with those found in the closely related etiobiliverdin-IVγ by X-ray crystallography: 5.3, 9.8, 11.0, 9.0, 22 and -2.0 degrees, respectively [23]. These differ from those found in biliverdin dimethyl ester by X-ray crystallography: 6.5, 11.8, 9.6, 2.0, 18.5 and 3.2 degrees, respectively, (in the same order as above), where there is less twisting about C(10)=C(11) [2,13]. In **3**, the corresponding torsion angles are -0.2, 31, -52, 0, -12 and -2.5 degrees, while in **4** they are -0.3, 32, -54, 0, -12 and -2.6 degrees for the minimum energy conformations [21]. Thus, **3** and **4** are found to be twisted about C(10) into a helical conformation with about the same pitch, 3.0 Å, as measured by the O(1) to O(19) vertical distance. In the parent verdin, the pitch is also computed to be 3.0 Å, with most of the distortion from a planar structure coming from rotations about C(5)-C(6) and C(14)-C(15).

Table 4

Influence of C(10) Substitution on Selected Molecular Parameters for Energy-Minimum Conformations of Octamethyl Rubins and Verdins Determined by Molecular Dynamics Calculations





Torsion and Interplanar Angles (°) and Nonbonded Distances (Å) for:	Parent R = H	**1** R = Ada	**2** R = *t*-Bu	Parent R = H	**3** R = Ada	**4** R = *t*-Bu
Φ_1 (22-9-10-11) [b]	-44	-39 (53) [g]	-38 (55) [g]	5	-52	-54
Φ_2 (9-10-11-23) [b]	79	74 (91) [g]	71 (91) [g]	2	-0.1	0.1
Ψ_1 (4-5-6-22) [c]	41	40 (35) [g]	40 (36) [g]	26	31	32
Ψ_2 (23-14-15-16) [c]	-35	-32 (-34) [g]	-32 (-35) [g]	26	-12	-12

Table 4 (continued)

Torsion and Angles (°) and Nonbonded Distances (Å) for:	Parent R = H	1 R = Ada	2 R = *t*-Bu	Parent R = H	3 R = Ada	4 R = *t*-Bu
(21-4-5-6) [d]	5.7	5.8 (3.3) [g]	5.6 (3.0) [g]	3.3	-0.2	-0.3
(14-15-16-24) [d]	-1.0	-1.1 (-2.9) [g]	-1.1 (-3.0) [g]	3.9	-2.5	-2.6
θ (dipyrrinone) [e]	30	31 (79) [g]	26 (120) [g]	10	36	34
θ (pyrrole) [e]	74	69 (107) [g]	67 (104) [g]	5	53	54
d(C=O ··· O=C) [f]	4.1	4.1 (12.8) [g]	4.0 (10.7) [g]	5.0	5.2	5.2
d(vertical)	3.1	3.1 (5.0) [g]	3.0 (2.2) [g]	2.9	3.0	3.0

[a] Using Sybyl ver. 6.0 for the Evans & Sutherland ESV-10$^+$ workstation, ref 8; [b] Values would be ~0° for the porphyrin conformation, ~60° for the ridge-tile conformation and ~180° for the linear conformation; [c] Indicates distortion from a planar dipyrrinone, where Ψ ~0°; [d] Indicates twist from 0° of C=C; [e] Interplanar dihedral angle using the average plane of each dipyrrinone or the dihedral angle of the two pyrroles adjacent to C(10); [f] Dipyrrinone oxygen-oxygen nonbonded distance and the vertical distance determined from one dipyrrinone oxygen to the average plane of the second dipyrrinone; [g] Parameters for nearest local minimum lying some 5 kcal/mole above the global minimum.

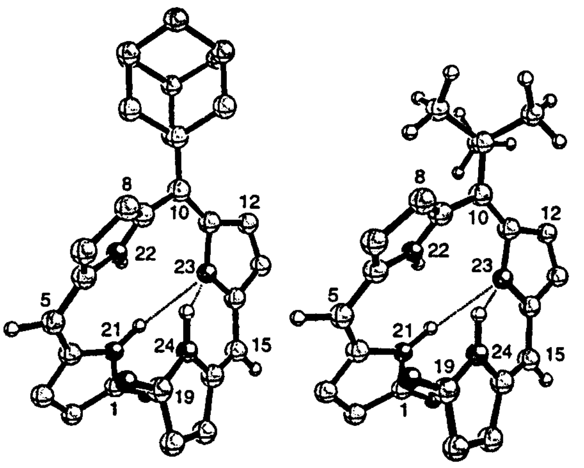


Figure 4. Ball and stick conformational representations for the helical global energy minimum conformations of verdins 3 and 4. Certain hydrogens and methyl groups are removed for clarity of representation. Intramolecular hydrogen bonds (N-H to N(23)) are shown in dashed lines.

In summary, bulky alkyl groups at C(10) in bilirubins and biliverdins can affect and stabilize conformation. 1-Adamantyl and *tert*-butyl groups appear to have similar effects in stabilizing a helical structure in rubins (**1** and **2**) and in opening up the helix of verdins (**3** and **4**).

EXPERIMENTAL

General Procedures.

All ultraviolet-visible spectra were recorded on a Perkin-Elmer λ-12 spectrophotometer. Nuclear magnetic resonance (nmr) spectra were obtained on GE QE-300 or GE GN-300 spectrometers operating at 300-MHz, or on a Varian Unity Plus 500-MHz spectrometer in chloroform-d solvent (unless otherwise specified). Chemical shifts were reported in δ ppm referenced to the residual chloroform ^{1}H signal at 7.26 ppm and ^{13}C signal at 77.0 ppm. A J-modulated spin-echo experiment (*Attached Proton Test*) was used to assign ^{13}C-nmr spectra. Melting points were taken on a Mel-Temp capillary apparatus and are uncorrected. Combustion analyses were carried out by Desert Analytics, Tuscon, AZ. Analytical thin layer chromatography was carried out on J. T. Baker silica gel IB-F plates (125 μ layers). Flash column chromatography was carried out using Woelm silica gel F. thin layer chromatography grade. Radial chromatography was carried out on Merck Silica Gel PF$_{254}$ with gypsum preparative layer grade, using a Chromatotron (Harrison Research, Inc., Palo Alto, CA). Spectral data were obtained in spectral grade solvents (Aldrich or Fisher). Adamantane, bromine, triethylamine, trifluoroacetic anhydride, pivaldehyde, 2,3-dichloro-5,6-dicyano-1,4-benzoquinone and trifluoroacetic acid were from Aldrich. Dichloromethane, methanol, dimethyl sulfoxide, formic acid, acetic acid, phosphorus pentoxide, and tetrahydrofuran were from Fisher.

10-(1-Adamantyl)-2,3,7,8,12,13,17,18-octamethyl-1,10,19,21,23,24-hexahydro-1,19-dioxobilin (1).

2,3,7,8-Tetramethyl-(10*H*)-dipyrrin-1-one (**5**) (432 mg, 2.00 mmoles) and adamantane-1-aldehyde (171 mg, 2.20 mmoles) were placed into a 25 ml round bottom flask along with a small magnetic stir bar. The flask was then fitted with a rubber septum and 10 ml of dichloromethane was added. After flushing the system with nitrogen for several minutes, trifluoroacetic acid (500 μl) was added *via* a syringe. Upon addition of the acid, the mixture became homogeneous and reddish orange. It was allowed to stir under nitrogen at room temperature for 5 hours; then the dark solution was taken up in 100 ml of dichloromethane and washed with water and saturated aqueous sodium chloride. After drying over anhydrous sodium sulfate, the solution was evaporated to give a dark solid. The crude dark condensation product was initially purified by flash-column chromatography on silica gel eluting with dichloromethane:methanol (100:2) to give an orange solid. Further purification was carried out by radial chromatography eluting with dichloromethane:methanol (100:2) to afford 364 mg (32%) of the desired yellow pigment **1**. An analytical sample was prepared by dissolving the sample in a minimum amount of hot dichloromethane and precipitating it out by the addition of methanol. The bright yellow pigment was filtered and dried over phosphorous pentoxide overnight. It had mp 290° dec; infrared (potassium bromide): ν 3380, 2906, 2849, 1668, 1499, 1444, 1347, 1311, 1262, 1222, 1173, 1111, 979, 945, 842, 816, 754, 731, 693, 664, 612, 569, 486 cm^{-1}; and ^{1}H-nmr and ^{13}C-nmr spectral data in Tables 1 and 2.

Anal. Calcd. for $C_{37}H_{46}N_4O_2$ (578.8): C, 76.78; H, 8.01; N, 9.68. Found: C, 76.42; H, 7.99; N, 9.51.

10-*tert*-Butyl-2,3,7,8,12,13,17,18-octamethyl-1,10,19,21,23,24-hexahydro-1,19-dioxobilin (2).

2,3,7,8-Tetramethyl-(10*H*)-dipyrrin-1-one (5) (432 mg, 2.00 mmoles) was coupled with pivaldehyde (118 µl, 1.00 mmoles) exactly as reported for 1 except the reaction time was increased to 8 hours. The crude dark condensation product was initially purified by flash-column chromatography on silica gel eluting with dichloromethane:methanol (100:1) to give an orange solid. Further purification was carried out by radial chromatography eluting with dichloromethane:methanol (100:1) to afford 223 mg (45%) of the desired yellow-orange pigment **2**. An analytical sample was prepared by the addition of methanol to a dichloromethane solution of the pigment followed by slow evaporation of the solvents under a stream of nitrogen. The resultant yellow crystals were filtered and dried over phosphorus pentoxide. The pigment had mp 270° dec; infrared (potassium bromide): ν 3422, 3377, 3283, 2920, 1697, 1678, 1653, 1630, 1438, 1364, 1251, 1178, 944 cm^{-1}; and ^{1}H-nmr and ^{13}C-nmr spectral data in Tables 1 and 2.

Anal. Calcd. for $C_{31}H_{40}O_2N_4$ (500.7): C, 74.35; H, 8.06; N, 11.20. Found: C, 74.31; H, 8.03; N, 11.08.

10-(1-Adamantyl)-2,3,7,8,12,13,17,18-octamethyl-1,19,21,24-tetrahydro-1,19-dioxobilin (3).

To a solution of **1** (57.1 mg, 0.100 mmole) in 30 ml of dry tetrahydrofuran was added an equivalent amount (0.100 mmole, 22.7 mg) of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone in 10 ml of dry tetrahydrofuran and the mixture was stirred at room temperature for 30 minutes. Upon addition of the quinone solution the rubin solution underwent an immediate color change from yellow to red. The red mixture was then poured into a two-phase system consisting of 100 ml of chloroform and 100 ml of 2% aqueous ascorbic acid then extracted with chloroform. (For better separation, saturated sodium chloride solution may be added.) The combined organic layers were washed with saturated aqueous sodium carbonate (2 x 100 ml), then with saturated aqueous sodium chloride (2 x 100 ml), and dried over anhydrous sodium sulfate. Removal of the solvents gave a reddish-purple solid which was purified by radial chromatography eluting with dichloromethane:methanol (100:2). This afforded 54.0 mg (95%) of the desired verdin **3** as a brick-red solid. An analytical sample was prepared by the addition of hexane to a dichloromethane solution of the pigment followed by slow evaporation of the solvents under a stream of nitrogen. The resultant bright red solid was collected by filtration and dried over phosphorus pentoxide in a drying pistol overnight. It had mp >340° dec; infrared (potassium bromide): ν 3438, 2907, 2849, 2355, 1708, 1663, 1637, 1448, 1394, 1261, 1250, 1170, 1133, 1108, 733, 688 cm^{-1}; and ^{1}H-nmr and ^{13}C-nmr in Tables 1 and 2.

Anal. Calcd. for $C_{37}H_{44}N_4O_2$ (576.8): C, 77.05; H, 7.69; N, 9.71. Calcd. for $C_{37}H_{44}N_4O_2 \cdot 1/2H_2O$ (585.8): C, 75.86; H, 7.57; N, 9.56. Found: C, 75.97; H, 7.84; N, 9.42.

10-*tert*-Butyl-2,3,7,8,12,13,17,18-octamethyl-1,19,21,24-tetrahydro-1,19-dioxobilin (4).

10-*tert*-Butylrubin (**2**) (50.0 mg, 0.100 mmole) was oxidized exactly as reported for **3**. The crude reddish-purple solid was purified by radial chromatography eluting with dichloromethane:methanol (100:2). This afforded 45.6 mg (95%) of the desired verdin **4** as a brick-red solid. It had mp >300° dec; infrared (potassium bromide): ν 3448, 3352, 2920, 2360, 1706, 1663, 1630, 1559, 1437, 1394, 1256, 1169, 1130, 1107, 934 cm^{-1}; and ^{1}H-nmr and ^{13}C-nmr in Tables 1 and 2.

Anal. Calcd. for $C_{31}H_{38}N_4O_2$ (498.7): C, 74.67; H, 7.68; N, 11.24. Found: C, 74.55; H, 7.48; N, 11.10.

2,3,7,8,12,13,17,18-Octamethyl-1,19,21,24-tetrahydro-1,19-dioxobilin.

The parent octamethylbilin was prepared by modification of Fischer and Aschenbrenner's method [24]. Dipyrrinone **5** (100 mg, 0.463 mmole) was mixed with 1 ml of aqueous formaldehyde (Aldrich) and 2 ml of concentrated hydrochloric acid then stirred for 10 minutes. Twenty milliliters of ice water was added to precipitate a green solid. The green material was purified by radial chromatography, eluting with dichloromethane:methanol (100:2). Evaporation of the solvents left a yellow-brown film, which was dissolved in a minimum amount of chloroform and precipitated by methanol to afford 20.8 mg (10%) of a yellow brown solid. It had mp 280° dec; infrared (potassium bromide): ν 3356, 2917, 1695, 1662, 1443, 1259, 1173, 942, 850, 757, 691 cm^{-1}; ^{1}H-nmr: δ 1.61 (s, 6H), 1.95 (s, 6H), 2.08 (s, 6H), 2.11 (s, 6H), 4.10 (s, 2H), 5.96 (s, 2H), 10.32 (s, 4H) ppm; ^{1}H-nmr (dimethyl-d$_6$ sulfoxide): δ 1.69 (s, 6H), 1.73 (s, 6H), 1.96 (s, 6H), 2.03 (s, 6H), 3.88 (s, 2H), 5.92 (s, 2H), 9.77 (s, 2H, NH), 10.27 (s, 2H, NH) ppm; ^{13}C-nmr (dimethyl-d$_6$ sulfoxide): δ 8.4 (q), 8.6 (q), 9.4 (q), 9.6 (q), 23.6 (t), 97.1 (d), 115.5 (s), 121.7 (s), 122.9 (s), 123.6 (s), 128.9 (s), 130.1 (s), 141.5 (s), 171.9 (s) ppm.

1-Bromoadamantane.

Adamantane was brominated as described in the literature [17]. It had mp 115-117° (lit 116-118°); ^{1}H-nmr: δ 1.71 (s, 6H), 2.09 (brs, 3H), 2.35 (d, 6H, J = 2.93 Hz.) ppm; ^{13}C-nmr: δ 32.6 (d), 35.6 (t), 49.3 (t), 66.3 (s) ppm.

1-Adamantanecarboxylic Acid.

1-Bromoadamantane was carboxylated using formic acid-sulfuric acid as described in the literature [18]. It had mp 178-180° (lit 181°); ^{1}H-nmr: δ 1.72 (d, 6H, J = 1.95 Hz), 1.91 (d, 6H, J = 2.93 Hz), 2.02 (brs, 3H), 10.75 (brs, 1H) ppm, ^{1}H-nmr (dimethyl-d$_6$ sulfoxide): δ 1.62 (s, 6H), 1.74 (s, 6H), 1.91 (s, 3H), 11.94 (brs, 1H) ppm; ^{13}C-nmr: δ 27.7 (d), 36.2 (t), 38.4 (t), 40.6 (s), 184.4 (s) ppm.

1-Adamantanemethanol.

1-Adamantanecarboxylic acid was reduced using lithium aluminum hydride according to the literature procedure [17]. It had mp 115-116° (lit 115°); ^{1}H-nmr: δ 1.72 (d, 6H, J = 1.95 Hz), 1.91 (d, 6H, J = 2.93 Hz.), 2.02 (brs, 3H), 10.75 (brs, 1H) ppm; ^{1}H-nmr (dimethyl-d$_6$ sulfoxide): δ 1.62 (s, 6H), 1.74 (s, 6H), 1.91 (s, 3H), 11.94 (brs, 1H) ppm; ^{13}C-nmr: δ 27.7 (d), 36.2 (t), 38.4 (t), 40.6 (s), 184.4 (s) ppm.

1-Adamantane Aldehyde.

To a dry 500 ml 3-neck round bottom flask equipped with a mechanical stirrer, dropping funnel, and thermometer was added dry dimethyl sulfoxide (80 mmoles, 6.2 g, 5.7 ml) diluted with dichloromethane (60 ml) and the solution was cooled below -60° with a dry ice-acetone bath. Next, trifluoroacetic anhydride (60 mmoles, 12.6 g, 8.4 ml), in 40 ml of dichloromethane was added with efficient stirring in about 10 minutes. After 10 minutes at or below -60°, a solution of 1-adamantanemethanol (40 mmoles, 6.6 g) in 40 ml of dichloromethane was added to the mixture in about 10 minutes. The rate of addition of alcohol was controlled to keep the temperature below -60°. Stirring was continued for an additional 5 minutes at -60°, then the cooling bath was removed and the stirring mixture was allowed to warm to room temperature. Next, triethylamine (16 ml) was added dropwise in about 10 minutes. The reaction mixture was then poured into a separatory funnel and washed with diluted hydrochloric acid, then with water and the combined aqueous washings were extracted once with dichloromethane. The combined organic extracts were dried over anhydrous sodium sulfate, and evaporated to afford a viscous oil which solidified under high vacuum. The solid weighed 6.0 g and gc-ms analysis showed it to be 78% pure. The product was purified by distillation under vacuum. It had ^{1}H-nmr: δ 1.73 (m, 12H), 2.06 (m, 3H), 9.32 (s, 1H) ppm; ^{1}H-nmr (dimethyl-d_6 sulfoxide): δ 1.64 (brs, 12H), 1.99 (brs, 3H), 9.25 (s, 1H) ppm; ^{13}C-nmr: δ 27.7 (d), 36.3 (t), 38.4 (t), 40.6 (s), 184.4 (s) ppm.

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