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Circular dichroism of distorted helices. C(10)-Adamantyl and C(10)-*tert*-butyl biliverdins

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Abstract

Bulky adamantyl and *tert*-butyl substituents at the central carbon of biliverdins cause the intrinsic verdin helical conformation to distort, and this results in a shift in pigment color from blue to red. Such distorted 'verdins' when derivatized as L-alanyl methyl ester bis-amides give modest circular dichroism Cotton effects. © 1998 Elsevier Science Ltd. All rights reserved.

1. Introduction

Biliverdin is a blue-green, water-insoluble pigment formed from heme during normal metabolism in plants and animals.[1–3] It occurs in avian eggshells, in the integument and hemolymph of insects, in the bile of some animals, and in the blood plasma of certain fish;[4] but it is not normally detectable in mammals because of its rapid enzymic reduction to the yellow pigment of jaundice, bilirubin, which is eliminated in normal metabolism by glucuronidation in the liver and excretion into bile.[1,2] In other animals, biliverdin is excreted directly. In blue-green algae, it is converted into photosynthetic pigments, phycoerythrobilin and phycocyanobilin.[1,3,5] In higher plants, it is converted into phytochrome, the photosensory pigment mediating photomorphogenesis.[3,5]

Though the constitutional structures of biliverdin and bilirubin are similar, their three-dimensional structures are not. Biliverdin and its naturally occurring analogs are conformationally flexible and in solution tend to adopt non-planar helical conformations (somewhat resembling a porphyrin when viewed down the helix axis) that are stabilized by intramolecular hydrogen bonding between NHs and the unprotonated N atom.[5,6] Bilirubin is also conformationally flexible in solution but, in contrast to biliverdin, adopts folded ridge-tile conformations that are stabilized by intramolecular hydrogen bonds between the pyrrole/lactam functions and the propionic carboxyl groups.[7] Similar ridge-tile conformations were thought to be sterically impossible for biliverdin because of the double-bonded

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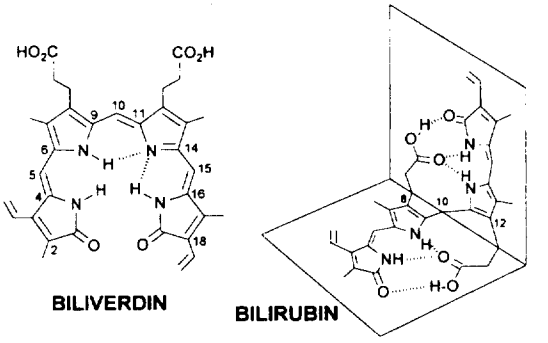
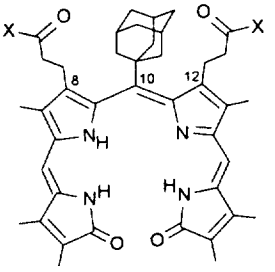
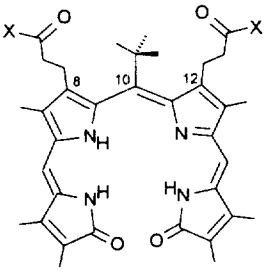
carbon at C(10). Recently, however, Falk et al.[8] showed that the presence of a bulky alkyl group (*tert*-butyl) at C(10) of a verdin analog, with only β-alkyl substituents on the four pyrrole rings, forces the helix to open rather widely. The torsion angle about the C(9)–C(10) bond was determined to be ~85°,[8] giving the verdin a ridge-tile shape without the benefit of the intramolecular hydrogen bonding characteristic of bilirubin. This conformational change is accompanied by a dramatic color change from the typical blue-green or blue verdin color to a deep red, a spectral shift attributable to decreased π-overlap through C(10) caused by a large increase in the pitch of the verdin helix.



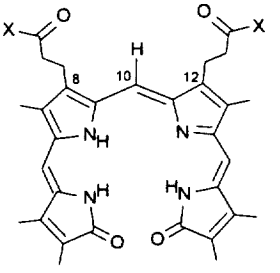
Intrigued by the possibility that C(10)-substituted verdins with propionic acid groups at C(8) and C(12) might adopt ridge-tile shapes that could be further stabilized by intramolecular hydrogen bonding, we synthesized biliverdin analogs **1** and **2** with bulky adamantyl and *tert*-butyl groups and compared their properties to those of the parent (**3**). Aware of earlier studies showing that biliverdin amides with L-alanine methyl ester exhibit unusually large circular dichroism Cotton effects,[9] we prepared such derivatives of **1**–**3**; namely **4**–**6**, to determine the influence of the pitch of the verdin helix on its chiroptical properties.



1: X= OH
4: X= L-Ala-OMe



2: X= OH
5: X= L-Ala-OMe



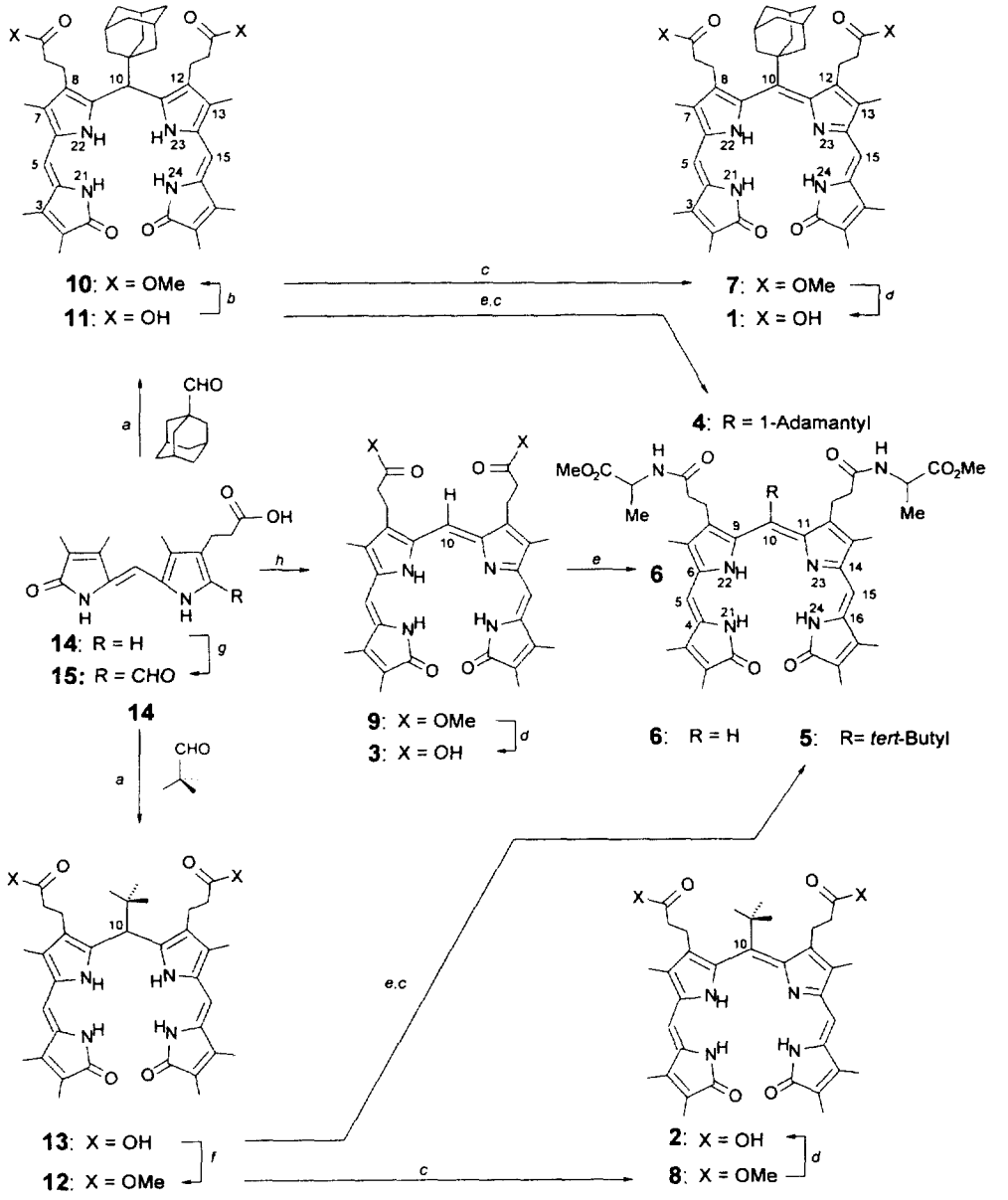
3: X= OH
6: X= L-Ala-OMe

2. Results and discussion

2.1. Synthesis

In an earlier study, C(10) adamantyl and *tert*-butyl rubins **11** and **13** (Scheme 1) had been prepared by condensing α-free dipyrrinone acid **14** with 1-formyl-adamantane or pivaldehyde and converting to their dimethyl esters (**10** and **12**).[10] Rubin dimethyl esters **11** and **12** were easily and directly converted to the corresponding verdin dimethyl esters (**7** and **8**) by treatment with DDQ. Consistent with the work of Falk et al.[8] on a *tert*-butyl verdin with no propionic acid or ester β-substituents, both **7** and **8** are red crystalline solids that give intense red solutions. They were saponified smoothly to afford pure, red

verdins **1** and **2**. In contrast, the parent verdin (**3**) and its dimethyl ester (**9**) are dark solids which give intense blue solutions. These were also synthesized from dipyrrinone **14**. α-Formylation of **14** with ethyl orthoformate and TFA gave **15**, and condensation of **15** with **14** afforded the verdin ester **9**.



Scheme 1. *a* TFA; *b* Cs_2CO_3/MeI/DMF; *c* DDQ; *d* NaOH/MeOH; *e* DPPA with L-Ala-OMe; *f* CH_2N_2; *g* $HC(OEt)_3$/TFA; *h* **14**+**15** in MeOH/HCl

Conversion of the previously prepared 10-adamantyl rubin acid **11** and 10-*tert*-butyl rubin acid **13** to their corresponding bis-amides of L-alanine methyl ester was accomplished smoothly and in good yields. Coupling[9,11] was achieved with L-alanine methyl ester hydrochloride using Shioiri's reagent,[12] diphenylphosphoryl azide (DPPA) in the presence of triethylamine, followed by oxidation using DDQ to

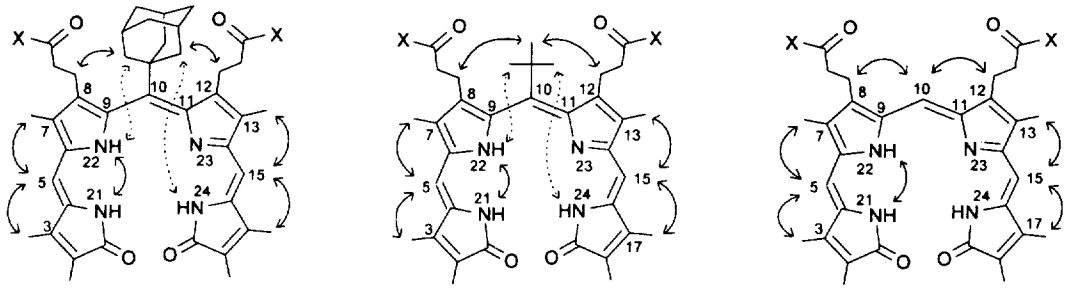


Figure 1. $^1H\{^1H\}$-Nuclear Overhauser effects in $(CD_3)_2SO$ as noted by double-headed arrows. Weak NOEs are shown as dashed lines. X=NHCH(Me)CO_2Me in **4**, **5** and **6**. X=OCH_3 in **7**, **8** and **9**

give the corresponding verdins **4** and **5**. The parent verdin bis-amide **6** was synthesized by coupling the parent verdin (**3**) with L-alanine methyl ester hydrochloride using DPPA.

2.2. *Nuclear Overhauser effects and conformation*

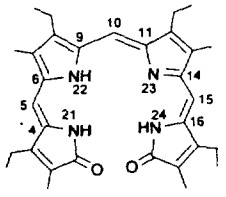
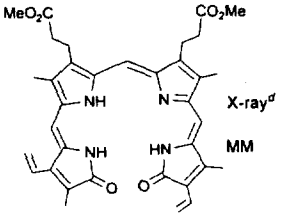
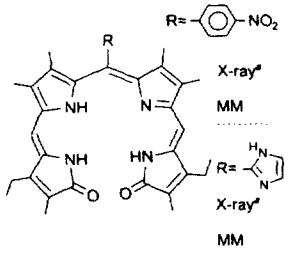
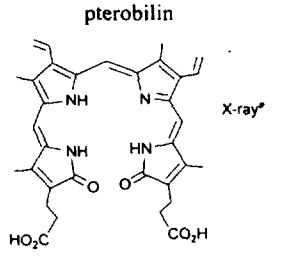
Biliverdins adopt the 4*Z*,10*Z*,15*Z* stereochemistry as the most stable configuration about the exocyclic carbon–carbon double bonds.[5] Supporting evidence for the porphyrin-like conformation in **4–9** comes from homonuclear $^1H\{^1H\}$-nuclear Overhauser effect (NOE) studies that reveal a synperiplanar (sp) or synclinal (sc) orientation within each dipyrrinone unit, about the C(4)–C(5), C(9)–C(10) and C(14)–C(15) carbon–carbon single bonds. Thus NOEs are seen between the C(5) and C(15) hydrogens and the methyls at C(3) and C(7), and at C(13) and C(17); NOEs are also seen between hydrogens at N(21) and N(22) (Fig. 1). In addition, NOEs are seen between the C(8′) and C(12′) methylenes (β-methylenes of the propionic chains) and the C(10) substituent: methyls in the case of **5** and **8**, ring methylenes from **4** and **7**, and the C(10)–H in **6** and **9**. The NOE data for the parent verdins (**6** and **9**) as well as the C(10) substituted verdins (**4**, **5**, **7**, **8**) suggest a *syn* geometry. In addition, in **4–8**, weak NOEs are found between the NHs and the C(10)-substituent. These data are consistent with twisting about the C(9)–C(10) and/or C(10)–C(11) bonds in order to bring the NHs closer to the C(10) substituent than is found in the parent verdins (**6** and **9**).

2.3. *Conformation from molecular dynamics calculation*

Insight into the preferred conformations of C(10)-substituted verdins and the influence of the size of the C(10) substituent may be obtained from molecular dynamics computations. Torsion angles about the carbon–carbon bonds linking the four rings are largely responsible for determining the verdin conformation and its helicity. Such torsion angles and helical pitch can be extracted from atomic coordinates of the minimum energy conformation determined by molecular dynamics calculations[5,13] and by X-ray crystallography.[5,14,15] Very few verdin crystal structures have been determined by X-ray methods. A comparison of the torsion angles obtained from both techniques for known verdins is shown in Table 1. Significantly, molecular mechanics calculations, which do not take into account crystal packing forces, reproduce the experimental data reasonably well and predict more twist in the dipyrrinones and less near C(10). Those verdins which have a C(10) imidazole or *p*-nitrophenyl substituent are found to have a larger pitch, by ~1 Å, over those without C(10) substituents.

The increase in helical pitch is the result of an enlarged torsion angle near the middle, about C(9)–C(10) rather than in the wings, C(5)–C(6) and C(14)–C(15). Only the biliverdin-IXγ (pterobilin) found within

Table 1
Comparison of conformation-determining torsion angles (°) and distances (d, Å) from X-ray crystallography[a] and molecular dynamics calculations[b]

Structure/Method	C=C 6-5-4-21	C-C 4-5-6-22	C-C 11-10-9-22	C=C 11-10-9-22	C-C 16-15-14-23	C=C 14-15-16-24	Distance[c] (O·····O)
 X-ray[d]	5.3	9.8	9.0	11.0	22.0	-2.0	(3.25)
MM	3.3	25.4	2.7	1.5	24.2	3.8	(3.1)
 X-ray[d]	3.2	18.5	9.6	2.0	11.8	6.5	(3.34)
MM	3.5	27.2	4.8	1.8	26.6	3.7	(3.0)
 X-ray[d]	11.0	10.9	14.8	6.4	14.0	2.2	(4.45)
MM	4.4	26.0	7.1	1.9	20.3	3.8	(3.0)
X-ray[e]	13.3	5.4	13.6	12.5	14.1	3.2	(4.31)
MM	4.4	20.2	14.5	1.6	17.5	3.4	(3.5)
pterobilin  X-ray[e]	-24	24	13	3	39	-13	(5.4)

[a] Data from X-ray diffraction atomic coordinates given in ref. 15.
[b] Data from energy minimum conformation determined by molecular mechanics calculations using SYBYL ver. 6.0 on an Evans & Sutherland ESV-10 workstation according to ref. 13.
[c] Distance (Å) between dipyrrinone lactam oxygens and (in parentheses) vertical distance between one dipyrrinone plane and the adjoining dipyrrinone's lactam oxygen.
[d] Data from X-ray diffraction atomic coordinates given in ref. 14.
[e] Data from X-ray diffraction atomic coordinates for biliverdin-XIIIγ in subunit D. The parameters for verdins in subunits A-C are not very different (ref. 15).

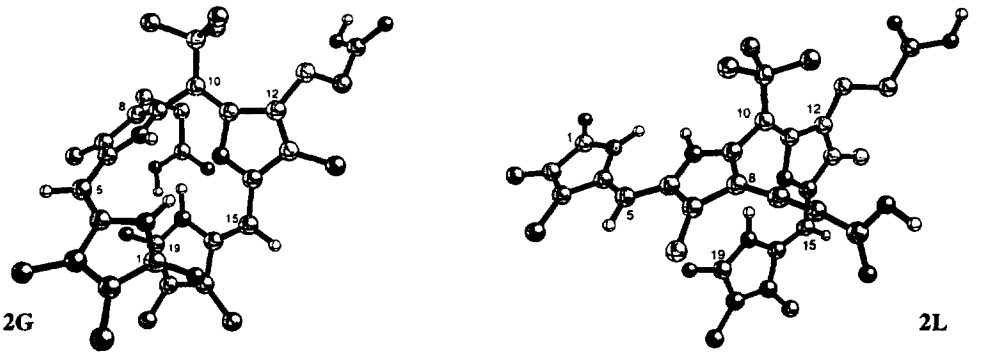


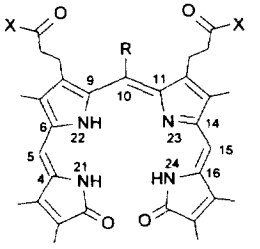
Figure 2. Ball and stick conformational drawings for the molecular dynamics computed[7,13] global minimum energy helical structure of (−sc, +sc, +sp)-**2** (**2G**, left) and its nearest local minimum (+sp, +ac, +sp)-helical structure of **2** (**2L**, right). Hydrogens on carbons are removed for clarity of presentation

the pocket of the bilin-binding protein from the butterfly *Pieris brassicae* shows a larger pitch (5.4 Å).[16] This pigment is bound (noncovalently) to the protein in a right-handed (*P*) helical conformation, with most of the increase in helical pitch coming from enlarged torsions at C(5)–C(6) and C(14)–C(15), as well as twisting about the adjoining carbon–carbon double bonds.

Although verdins with NHs and hydrogens at carbons 5, 10 and 15 are generally known (Table 1) to adopt a helical conformation with synperiplanar (sp) stereochemistry at the 5–6; 9–10 and 14–15 carbon–carbon single bonds,[5] the presence of a bulky alkyl group at C(10) raises the spectre that an anticlinal (ac) or antiperiplanar (ap) configuration might be energetically preferred over sp or synclinal (sc). However, for C(9)–C(10) ac stereochemistry in **4**–**8**, we compute (molecular dynamics, Sybyl[7,13]) a local minimum some 9 kcal/mole higher in energy than the global minimum sc (Fig. 2). Minimal intramolecular hydrogen bonding in the global minimum adds ~2 kcal/mole stabilization without altering conformation. Since very little intramolecular hydrogen bonding is evident in either the (−sc, +sc, +sp) global minima of **1**–**6**, or in the (+sp, +ac, +sp) local minima, the former appears to be energetically favored on the basis of having minimized nonbonded steric effects. Such large energy differences suggest a strong preference for the sc conformation about C(9)–C(10) and the presence of very little of the ac conformation.

As above, certain important torsion angles in verdins can be used as a gauge of distortion from co-planarity, especially about the C(5)–C(6), C(14)–C(15) and C(9)–C(10) single bonds. The first two angles measure distortion from a planar dipyrrinone; the last gives a measure of co-planarity of the two dipyrrinones. Taken collectively, such torsion angles can give one a measure of helicity in the verdin. Molecular dynamics calculations for the C(10) adamantyl (**1**, **4**, **7**) and *tert*-butyl (**2**, **5**, **8**) verdins of this work indicate considerably more twisting about the C(9)–C(10) single bond than in the parent verdins (**3**, **6**, **9**). In the C(10)-substituted verdins the C(11)–C(10)–C(9)–N(22) torsion angles are large (Table 2), indicating that the component dipyrrinones are rotated ~60° out of co-planarity into an sc conformation from the sp of parents, where the torsion angles are quite small (~5°). For the C(10)-substituted verdins, additional large torsion angles (~40°) are found about the C(5)–C(6) bond of the dipyrrinone attached to C(10) by a single bond; whereas smaller distortions (~25°) from co-planarity are found about the C(14)–C(15) bond in the dipyrrinone attached to C(10) by a double bond. In contrast, torsion angles about the same bonds in the parent verdins (**3**, **6**, **9**) indicate nearly equal twisting (23–27°) within each dipyrrinone. With much less distortion from co-planarity at C(10), most of the helicity in the parents comes from twisting in the dipyrrinone wings rather than the center. These computed data for the unsubstituted verdins are comparable to those found in other verdins (Table 1) with a hydrogen or non-

Table 2
Conformation-defining torsion angles obtained for global minimum conformations of verdin acids (**1**, **2**, **3**: X=OH), esters (**7**, **8**, **9**: X=OCH_3) and amides (**4**, **5**, **6**: X=L-Ala-OMe) by molecular dynamics calculations[a]



Torsion Angle (°)	R= 1-Adamantyl			R= *tert*-Butyl				R= H		
or Distance (d,Å)	**1**	**4**	**7**	**2G**[b]	**2L**[b]	**5**	**8**	**3**	**6**	**9**
C=C 6-5-4-21	-0.8	0.0	-1.5	0.8	0.7	-0.2	-1.5	3.1	3.8	3.7
C=C 14-15-16-24	3.6	5.1	5.0	3.5	0.1	5.5	5.0	3.7	3.6	3.4
C-C 4-5-6-22	-40	-38	-40	-40	11	-38	-40	27	25	25
C-C 16-15-14-23	23	16	25	23	6.2	15	25	27	23	23
C-C 11-10-9-22	60	62	60	60	82	63	60	4.4	4.9	4.5
C=C 9-10-11-23	-2.8	-4.9	-3.5	-2.3	2.4	-3.6	-3.4	1.3	0.8	1.2
Distance (O·····O)	5.4	5.1	5.4	5.4	6.8	5.1	5.4	5.1	4.4	4.9
Distance (O·····O)[c]	3.7	3.0	3.7	3.6	5.5	3.2	3.7	3.2	3.0	2.7

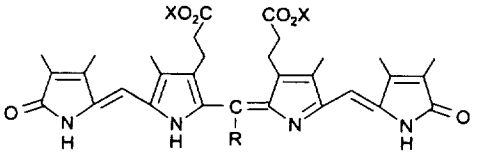
[a] Using SYBYL ver. 6.0 on an Evans & Sutherland ESV-10$^+$ workstation according to ref. 7. [b] **2G** = global minimum of **2**; **2L** = local minimum of **2**. [c] Vertical distance between dipyrrinone plane to remaining dipyrrinone oxygen.

bulky substituents at C(10). The data of Table 2 clearly suggest that the presence of bulky C(10) alkyl substituents forces the verdin helix to distort considerably, with enlarged torsion angles at C(5)–C(6), C(9)–C(10) and C(14)–C(15). Falk et al.[8] have suggested that the two dipyrrinones are orthogonal, which in **2** appears to correspond to the nearest local minimum (**2L**, Table 2) lying ~9.5 kcal/mole higher than the global minimum (**2G**). In **2L**, there is far less distortion from planarity within the dipyrrinones, but the torsion about the C(9)–C(10) bond is larger and the helix is open more widely. Our results from molecular dynamics calculations indicate an interplanar angle somewhat smaller than 90°.

2.4. *Conformation from UV–vis spectroscopy*

Verdins with C(10) *tert*-butyl and adamantyl groups are not blue or blue-green; they are red. This is because the bulky alkyl groups force the helical structure of the pigment to open widely, which decreases π-overlap between the two dipyrrinones through C(10). In Falk's 3,7-diethyl-2,7,8,12,13,18-hexamethyl-10-*tert*-butyl-1,19,21,24-tetrahydro-23*H*-bilin-1,19-dione (an analog of **2**), the two dipyrrinones are predicted to be almost orthogonal.[8] The C(10) adamantyl (**1** and **7**) and *tert*-butyl (**2** and **8**) verdin acids and dimethyl esters exhibit very different UV–vis spectra from their parents (**3** and **9**) (Table 3). The long wavelength absorption λ_{max} is strongly hypsochromically shifted—corresponding to a shift in color from blue to red. Interestingly, there is a strong solvatochromic effect on the long-wavelength λ_{max} (*cf.* $(CH_3)_2SO$ solvent to $CHCl_3$). Whether this is due to the more polar solvent disrupting dimer formation[5] is unclear.

Table 3
Solvent dependence of UV–visible spectra[a] of verdin acids (X=H): **1** (R=1-adamantyl), **2** (R=*tert*-butyl), **3** (R=H) and their corresponding dimethyl esters (X=CH_3): **7–9**



Solvent	Acids			Dimethyl Ester		
	1	**2**	**3**	**7**	**8**	**9**
C_6H_6	27100 (404) 5500 (495)	24500 (405) 7100 (488)	37700 (370) 14300 (636)	32815 (393) 37680 (411) 6960 (486)	32503 (398)^{sh} 36151 (413) 14572 (464)	 50000 (368) 22600 (658)
CH_2Cl_2	26500 (402) 4480 (494)	26500 (404) 6250 (487)	34900 (365) 10200 (630)	35393 (390) 37787 (404) 7405 (501)	28660 (389)^{sh} 31730 (408) 15270 (465)	 55900 (365) 16400 (634)
$CHCl_3$	26100 (404) 4200 (500)	28500 (405) 8000 (497)	34800 (368) 10600 (630)	33792 (390) 35966 (404) 8185 (497)	41308 (394) 30245 (410)^{sh} 16711 (460)^{sh}	 53100 (367) 15100 (636)
$(CH_3)_2CO$	29700 (398) 4580 (509)	29600 (403) 5700 (483)	33200 (361) 12300 (623)	40755 (400) 6540 (515)	39245 (390)^{sh} 40893 (404) 6296 (516)	 55900 (364) 16500 (626)
CH_3OH	 31300 (401) 3860 (511)	29500 (391)^{sh} 30900 (403) 5430 (485)	 37000 (364) 11100 (647)	38966 (385) 43680 (404) 6798 (508)	39579 (389)^{sh} 44258 (410) 6101 (507)	 54400 (362) 15300 (641)
$(CH_3)_2SO$	28600 (388) 30200 (405) 3760 (536)	 28000 (397) 3660 (530)	 35100 (371) 12600 (640) 11800 (685)^{sh}	39781 (389) 43247 (410) 6994 (537)	38686 (385)^{sh} 41748 (414) 4201 (538)	 55200 (371) 17800 (633)

[a] ϵ^{max} and ϵ^{sh} in L · mol^{-1} cm^{-1} at (λ in nm) for 10^{-5} M solutions.

2.5. *Circular dichroism and stereochemistry*

Biliverdin adopts either of two enantiomeric lock-washer shapes as the most stable conformation (Fig. 4).[5] The interconversion barrier (Fig. 3) separating these (***M*** and ***P***) helical conformational enantiomers is rather low ($\Delta G^{\ddagger}$ ~10 kcal/mole),[17] even when the two propionic acid groups are linked as a diester,[13] but it can be raised to $\Delta G^{\ddagger}_{293}$~21–24 kcal/mole by linking carbons 2 and 18 with a 4-carbon butenyl bridge.[18] The ***M*** ⇌ ***P*** conformational equilibrium in unbridged verdins may be tilted toward the ***M*** or the ***P*** helix by the constraints of protein binding, as in the pterobilin (Table 1) found noncovalently attached in the bilin binding protein from *Pieris brassicae*[5,16] or in biliverdin bound noncovalently to human serum albumin.[19] It may also be tilted by covalently attaching a chiral group, as in biliverdin, with its propionic acids derivatized as amides with optically active amino acid esters or peptide esters.[9] Amides with L-alanine methyl ester and with the tripeptide L-Ala-L-Ala-L-Ala methyl ester were shown previously to exhibit especially strong diastereoselectivity for the ***P*** helical verdin conformer.[9]

Circular dichroism (CD) spectra of the bis-amides (**4–6**) of L-alanine methyl ester with verdins **1–3** are shown in Figs. 4–7. Bisignate CD curves are found for the UV–vis transitions near 430 nm of the

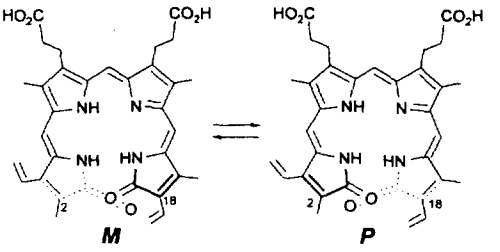


Figure 3. Interconverting *M* and *P* helicity biliverdin

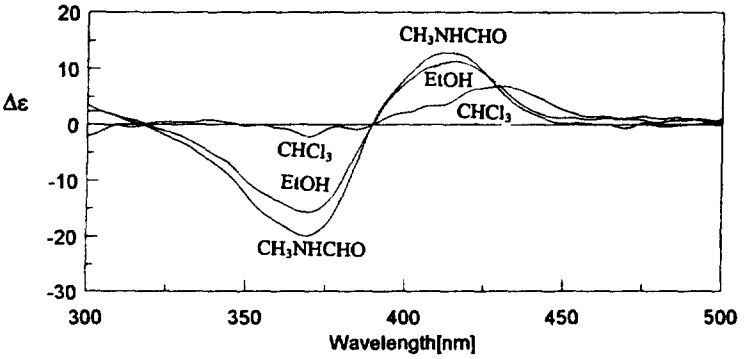


Figure 4. Solvent dependence of the circular dichroism spectra of C(10)-adamantyl verdin bis-amide **4** run at 22°C in 2.6×10^{-5} M concentration in $CHCl_3$, EtOH and CH_3NHCHO

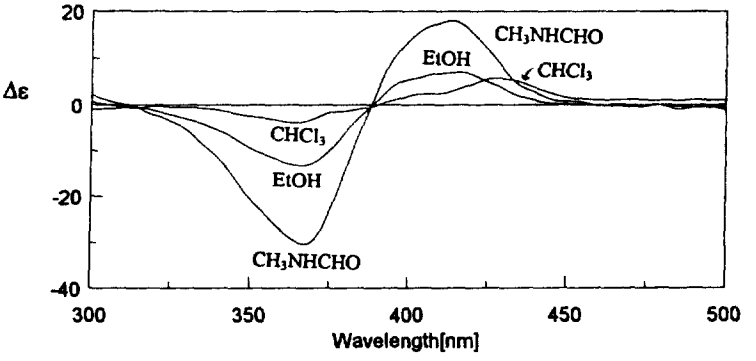


Figure 5. Solvent dependence of the circular dichroism spectra of C(10)-*tert*-butyl verdin bis-amide **5** run at 22°C in 2.7×10^{-5} M concentration in $CHCl_3$, EtOH and CH_3NHCHO

C(10)-*tert*-butyl (**4**) and C(10)-adamantyl (**5**) verdin amides, Figs. 4 and 5, respectively. The ~500 nm, weak UV–vis transition (Table 3) appears to be CD inactive. In contrast, the parent verdin (**6**) exhibits monosignate CD curves near 390 and 630 nm, which correspond approximately to the 400 and 500 nm transitions of **4** and **5** (Table 3). The bisignate curves seen for **4** and **5** are reminiscent of those found for the ~430–450 nm transition of bilirubin and mesobilirubin.[7,20] Noticeably, the CD Cotton effects found for **4** and **5** (Figs. 4 and 5) are far weaker in the less polar solvents, rather than the more polar (Table 4); the opposite of that seen in the parent (**6**) (Fig. 6). As expected, the Cotton effects of **6** are quite similar in magnitude to those of the bis-amide of biliverdin-IXα (Table 4.) Even in $(CH_3)_2SO$ solvent, **4** and **5** maintain strong bisignate Cotton effects, while those of **6** are very weak (Fig. 7).

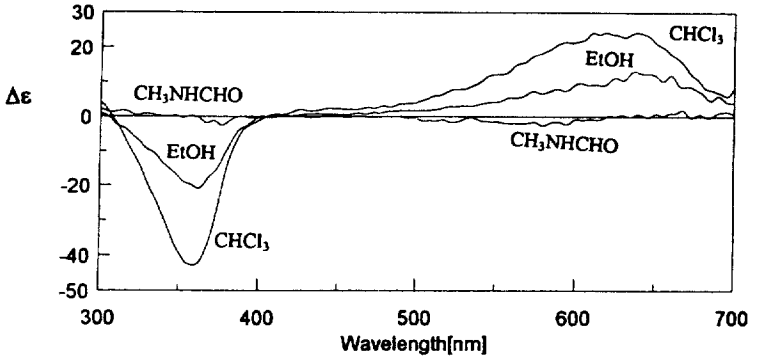


Figure 6. Solvent dependence of the circular dichroism spectra of parent verdin **6** run at 22°C in 2.6×10^{-5} M concentration in $CHCl_3$, EtOH and CH_3NHCHO

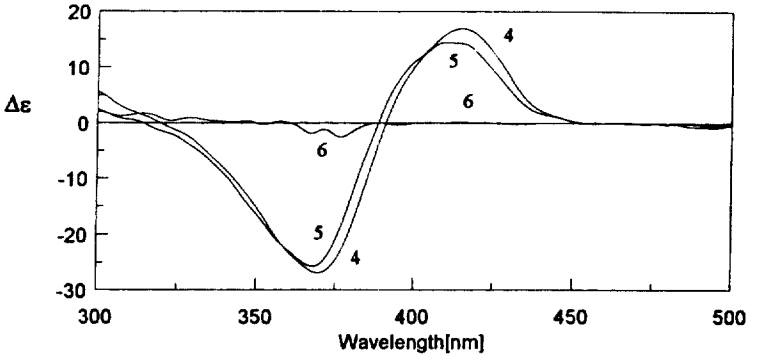


Figure 7. Comparison of the circular dichroism spectra of verdin bis-amides **4–6** in $(CH_3)_2SO$ solvent at 22°C. Pigment concentrations are $\sim2.6\times10^{-5}$ M

3. Concluding comments

The presence of sterically demanding alkyl groups, such as *tert*-butyl and 1-adamantyl at the center (or C(10)) of the biliverdin structure, as in **1** and **2**, induces significant conformational deformation: from a helical shape which is relatively flat in the center to a more open helical shape that is bent some 60° at the middle (Table 2). This causes **1** and **2** to be red in color rather than blue (**3**). Such a dramatic color change was noted previously by Falk et al.,[5,8] who first found that a C(10)-*tert*-butyl group produces a more open helix in a verdin without propionic acid groups. Our verdins (**1** and **2**), which are twisted about the center to give helical conformations similar to those found in bilirubin,[7] show very little tendency to engage in the intramolcular hydrogen bonding so dominating in the bilirubin three-dimensional structure. In **1** and **2**, the $M \rightleftharpoons P$ interconversion barrier (15 kcal/mole) is ~50% larger than that of the parent, as determined by molecular dynamics calculations. The increase is apparently due to nonbonded steric repulsions between the *tert*-butyl group and the $C(8^1)/C(12^1)$ CH_2 groups at the interconversion transition state. The (−sc, +sc, +sp) conformation in **1** and **2** is a global minimum; the (+sp, +sp, +sp) is a global minimum in the parent (**3**). Local minima for **1** and **2** (+sp, +ac, +sp) lie some 9 kcal/mole higher in energy.

Table 4
Comparison of solvent dependence of circular dichroism and UV–visible spectral data of verdin bis-amides **4–6** with biliverdin-IXα

Solvent	Verdin bis-amide with *S*-(+) alanine methyl ester	Circular Dichroism			UV-Visible		
		$\Delta\epsilon_{max}$ (λ_1)	λ at Δε=0	$\Delta\epsilon_{max}$ (λ_2)	ϵ_1 (λ_1)	ϵ_2 (λ_2)^{sh}	ϵ_3 (λ_3)
C_6H_6	10-(1-Adamantyl) **4**	+15 (430)	393	-19 (370)	36300 (415)	32550 (398)	5900 (508)
	10-tert-Butyl **5**	+20 (427)	390	-31 (367)	41050 (413)	37300 (398)	7200 (505)
	Parent **6**	+37 (634)	—	-63 (361)	53150 (367)	—	17600 (627)
	Biliverdin-IXα[a]	+39 (657)	—	-57 (378)	51100 (380)	—	15500 (653)
$CHCl_3$	10-(1-Adamantyl) **4**	+6.8 (430)	390	-2.3 (369)	41200 (410)	—	7000 (514)
	10-tert-Butyl **5**	+5.5 (428)	389	-4.2 (364)	41250 (407)	—	7780 (514)
	Parent **6**	+24 (619)	—	-43 (392)	57000 (367)	—	16700 (630)
	Biliverdin-IXα[a]	+32 (657)	—	-47 (386)	53450 (378)	—	14600 (658)
CH_2Cl_2	10-(1-Adamantyl) **4**	+5.9 (434)	390	-1.1 (366)	36800 (410)	—	6200 (517)
	10-tert-Butyl **5**	+9.5 (427)	388	-9.1 (364)	42900 (407)	—	7850 (514)
	Parent **6**	+21 (632)	—	-35 (360)	56600 (366)	—	16300 (627)
	Biliverdin-IXα[a]	+22 (658)	—	-33 (378)	52400 (378)	—	13950 (658)
CH_3CH_2OH	10-(1-Adamantyl) **4**	+11 (430)	390	-16 (369)	46100 (413)	40550 (390)	6400 (514)
	10-tert-Butyl **5**	+8.2 (443)	389	-14 (366)	45600 (406)	39200 (382)	7650 (512)
	Parent **6**	+13 (637)	—	-21 (362)	55450 (365)	—	15600 (639)
	Biliverdin-IXα[a]	+15 (663)	—	-20 (377)	51600 (377)	—	14450 (665)
THF	10-(1-Adamantyl) **4**	+2.1 (412)	390	-3.0 (364)	35900 (405)	—	5350 (526)
	10-tert-Butyl **5**	+3.0 (407)	384	-5.0 (362)	41700 (399)	—	6800 (522)
	Parent **6**	+8.0 (635)	—	-14 (361)	52150 (367)	—	17050 (622)
	Biliverdin-IXα[a]	+7.5 (660)	—	-14 (378)	54500 (378)	—	16800 (645)
$(CH_3)_2SO$	10-(1-Adamantyl) **4**	+17 (415)	391	-27 (370)	46500 (416)	42300 (394)	6250 (543)
	10-tert-Butyl **5**	+15 (410)	389	-26 (369)	47400 (411)	42200 (387)	7100 (540)
	Parent **6**	+1.8 (666)	—	-2.6 (377)	57100 (371)	—	18100 (632)
	Biliverdin-IXα[a]	NA	—	NA	NA	—	—
N-methyl formamide	10-(1-Adamantyl) **4**	+13 (437)	390	-20 (369)	44800 (413)	40200 (390)	6750 (525)
	10-tert-Butyl **5**	+18 (440)	389	-31 (368)	48200 (407)	44000 (389)	7850 (527)
	Parent **6**	+1.2 (663)	390	-1.8 (376)	55600 (372)	—	17090 (630)
	Biliverdin-IXα[a]	NA	—	NA	NA	—	—

[a] Data taken from Lehner *et al. J. Chem. Soc. Perkin Trans 2*, **1985**, 421.

4. Experimental

4.1. General procedures

All UV–visible spectra were recorded on a Perkin–Elmer λ-12 spectrophotometer, and all circular dichroism (CD) spectra were recorded on a Jasco J-600 instrument. 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 $CDCl_3$ solvent (unless otherwise specified). Chemical shifts were reported in δ ppm referenced to the residual $CHCl_3$ ^1H 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, Tucson, 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). HPLC analyses were carried out on a Perkin–Elmer Series 4 high performance liquid chromatograph with an LC-95 UV–visible spectrophotometric detector (set at 410 nm) equipped with a Beckman–Altex ultrasphere-IP 5 μ C-18 ODS column (25×0.46 cm) and a Beckman ODS precolumn (4.5×0.46 cm). The flow rate was 1.0 ml/min, and the elution solvent was 0.1 M di-*n*-octylamine acetate in 5% aqueous methanol pH 7.7, 31°C. Spectral data were obtained in spectral grade solvents (Aldrich or Fisher). Trifluoroacetic acid, triethyl orthoformate, diphenylphosphorylazide, 2,3-dichloro-3,4-dicyano-1,6-benzoquinone (DDQ) and L-alanine methyl ester hydrochloride were from Aldrich. Dichloromethane, methanol, acetic acid, phosphorus pentoxide, and tetrahydrofuran were from Fisher.

4.2. 10-(1-Adamantyl)-8,12-bis-(2-methoxycarbonylethyl)-2,3,7,13,17,18-hexamethyl-(21H,24H)-bilin-1,19-dione (7)

To a solution of 10-adamantyl rubin dimethyl ester **10**[10] (71.6 mg, 0.100 mmol) in 30 ml of dry THF was added an equivalent amount (0.100 mmol, 22.7 mg) of DDQ in 10 ml of dry THF, and the mixture was stirred at room temperature for 30 min. Upon addition of the DDQ 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 $CHCl_3$ and 100 ml of 2% aq. ascorbic acid then extracted with $CHCl_3$. (For better separation, satd NaCl solution may be added.) The combined organic layers were washed with satd aq. Na_2CO_3 (2×100 ml), then with satd aq. NaCl (2×100 ml), and dried over anhydrous Na_2SO_4. Removal of the solvents gave a brick-red solid. The crude product was purified by radial chromatography eluting with 100:3 (by vol) dichloromethane:methanol. This afforded 54.9 mg (77%) of the desired verdin (**7**) as a reddish-purple 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 P_2O_5 in a drying pistol overnight. It had mp of 295°C (dec.); IR (KBr) ν: 3423, 2907, 2360, 1736, 1708, 1664, 1636, 1560, 1508, 1438, 1364, 1260, 1168, 1109, 758, 691, 577 cm^{-1}; ^1H NMR ($(CD_3)_2SO$) δ: 1.69 (brs, 6H, γ-adamantane), 1.75 (s, 3H), 1.81 (s, 3H), 1.92 (s, 3H), 1.97 (s, 3H), 2.05 (brs, 9H, 3H, β-adamantane +6H, pyrrole CH_3), 2.07 (s, 3H), 2.13 (s, 6H, α-adamantane), 2.30 (t, 4H, J=6.83 Hz), 2.42 (t, 4H, J=6.83 Hz), 3.30 (s, 3H, OCH_3), 3.50 (s, 3H, OCH_3), 5.91 (s, 1H), 6.04 (s, 1H), 9.92 (s, 1H, NH), 10.35 (s, 1H, NH), 10.46 (s, 1H, NH) ppm. ^{13}C NMR ($(CD_3)_2SO$) δ: 8.72 (q), 8.78 (q), 9.70 (q), 9.75 (q), 9.83 (q), 9.94 (q), 20.81 (t), 29.32 (d), 34.35 (t), 36.83 (t), 42.03 (s), 42.68 (t), 51.27 (q), 51.74

(q), 95.79 (d), 97.40 (d), 121.22 (s), 122.45 (s), 124.29 (s), 124.74 (s), 129.68 (s), 130.42 (s), 130.57 (s), 136.36 (s), 141.48 (s), 141.93 (s), 143.68 (s), 146.87 (s), 150.22 (s), 155.41 (s), 166.86 (s), 171.73 (s), 172.34 (s), 172.46 (s), 172.96 (s) ppm. UV–vis in Table 3. Anal. calcd for $C_{43}H_{52}N_4O_6$ (720.9): C, 71.64; H, 7.27; N, 7.77. Found: C, 71.34; H, 7.57; N, 7.56.

4.3. 10-(1-Adamantyl)-8,12-bis-(2-carboxyethyl)-2,3,7,13,17,18-hexamethyl-(21H,24H)-bilin-1,19-dione (1)

Dimethyl ester **7** (34 mg, 0.05 mmol) and 20 mg of ascorbic acid were dissolved in 26 ml of 1:1 THF:MeOH (by vol). Next, 26 ml of 0.10 M NaOH was added, and the mixture was stirred at 37–40°C for 90 min. The cooled basic solution was acidified with glacial acetic acid at 0°C, and extracted with $CHCl_3$ until no color remained in the aqueous layer. The organic layer was dried over anhyd. Na_2SO_4. Removal of the solvents left a red residue which had low solubility in most common organic solvents. It was dissolved in dichloromethane:methanol and purified by radial chromatography. Elution with 95:4:1 (by vol) dichloromethane:methanol:acetic acid afforded 26 mg (80%) of the desired diacid (**1**). It had mp of 260°C (dec.); IR (KBr) ν: 3422, 2909, 1708, 1685, 1636, 1450, 1396, 1343, 1261, 1171, 1109, 945, 757, 578 cm^{-1}; ^1H NMR ($(CD_3)_2SO$) 75°C δ: 1.72 (d, 6H, J=12.0 Hz, γ-adamantane), 1.83 (s, 6H), 1.85 (m, 3H, β-adamantane), 2.00 (s, 6H), 2.09 (s, 6H), 2.62, (m, 14H, 8H, CH_2CH_2)+(6H, α-adamantane)] 6.00 (brs, 2H), 9.72 (brs, 1H, NH), 10.29 (brs, 2H, NH), 12.00 (brs, 2H, CO_2H) ppm. ^{13}C NMR ($(CD_3)_2SO$) 75°C δ: 7.75 (q), 8.34 (q), 19.99 (t), 27.88 (d), 33.69 (t), 35.85 (t), 40.96 (s), 41.25 (t), 100.08 (d), 112.25 (s), 128.53 (s), 140.06 (s), 143.26 (s), 149.00 (s), 150.11 (s), 166.54 (s), 172.13 (s), 173.87 (s) ppm. UV–vis in Table 3. Anal. calcd for $C_{41}H_{48}N_4O_6$ (692.9): C, 71.08; H, 6.98; N, 8.09. Calcd for $C_{41}H_{48}N_4O_6 \cdot H_2O$ (710.9): C, 69.27; H, 7.08; N, 7.68. Found: C, 69.57; H, 7.02; N, 7.31.

4.4. 10-(1-Adamantyl)-8,12-bis-(2-carboxyethyl)-2,3,7,13,17,18-hexamethyl-1,19,21,24-tetrahydro-1,19-dioxobilin bis-amide with S-(+)-alanine methyl ester (4)

10-Adamantyl rubin bis-amide[10] (62 mg, 0.072 mmol) was placed into a 100 ml round bottom flask and dissolved in 30 ml of dry THF. A solution of an equimolar amount of DDQ (16.3 mg) dissolved in 10 ml of dry THF was added, and the mixture was stirred at room temperature for 30 min. The red solution was poured into a two-phase system of 100 ml of $CHCl_3$ and 100 ml of 2% aq. ascorbic acid. It was then worked up exactly as for verdin dimethyl ester **7**. The crude product was purified by radial chromatography eluting with 100:4 (by vol) dichloromethane:methanol. The red residue was dissolved in a minimum amount of CH_2Cl_2 and precipitated by the addition of hexane. This afforded 22 mg (35%) of the desired verdin (**4**) as a reddish-purple solid. It had mp of 280°C (dec.); IR (KBr) ν: 3433, 2910, 2845, 2355, 1736, 1665, 1627, 1534, 1447, 1387, 1360, 1338, 1256, 1218, 1169, 1104, 1082, 1055, 979, 940 cm^{-1}; UV–vis: ε (λ^{max}, nm) [benzene: 36,280 (415), 32,550 (398)^{sh} 5900 (508)]; [CH_2Cl_2: 36,800 (410), 6190 (517)]; [$HCONH(CH_3)$: 44,780 (413), 40,230 (390)^{sh}, 6750 (527)]; [$CHCl_3$: 41220 (410), 7010 (514)]; [THF: 35,900 (405), 5360 (526)]; [CH_3CH_2OH: 46120 (413), 40550 (390)^{sh}, 6410 (514)]; [$(CH_3)_2SO$: 46530 (416), 42300 (394)^{sh}, 6260 (543)]; ^1H NMR $(CD_3)_2SO$ δ: 1.09 (d, 1.5H, J=7.3 Hz), 1.15 (d, 1.5H, J=7.3 Hz), 119 (m, 6H, adamantane γ Hs), 1.20 (d, 1.5H, J=7.3 Hz), 1.21 (d, 1.5H, J=7.3 Hz), 1.74 (s, 3H), 1.82 (s, 3H), 1.91 (s, 3H), 2.04 (s, 3H), 2.05 (s, 1.5H), 2.06 (s, 1.5H), 2.08 (s, 3H), 2.13 (m, 6H, adamantane α Hs), 2.17 (m, 4H), 2.21 (m, 4H), 3.53 (s, 1.5H), 3.54 (s, 1.5H), 3.546 (s, 1.5H), 3.551 (s, 1.5H), 4.10 (m, 2H), 5.93 (s, 1H), 6.05 (s, 1H), 7.69 (d, 0.5H, J=7.3 Hz, CONH), 7.75 (d, 0.5H, J=7.3 Hz, CONH), 8.26 (d, 1H, J=6.8 Hz, CONH), 9.89 (brs, 1H, NH), 10.29 (s, 0.5H, NH), 10.32 (brs, 0.5H, NH), 10.49 (brs, 1H, NH) ppm; ^{13}C NMR ($(CD_3)_2SO$) δ: 173.56 (s), 172.40 (s), 172.00 (s), 171.79

(s), 17.46 (s), 171.30 (s), 167.14 (s), 156.23 (s), 150.82 (s), 145.94 (s), 144.72 (s), 142.01 (s), 141.63 (s), 136.30 (s), 134.59 (s), 131.23 (s), 130.77 (s), 130.72 (s), 125.12 (s), 124.65 (s), 123.49 (s), 122.64 (s), 122.00 (s), 97.61 (d), 95.98 (s), 48.01 (q), 47.52 (q), 42.70 (t), 41.99 (s), 36.51 (t), 35.46 (t), 32.16 (d), 21.42 (t), 21.35 (t), 17.59 (q), 17.40 (q), 17.31 (q), 9.96 (q), 9.92 (q), 9.88 (q), 9.79 (q), 8.75 (q), 8.56 (q), 8.35 ppm. Anal. calcd for $C_{49}H_{62}N_6O_8$ (863.1): C, 68.19; H, 7.24; N, 9.74. Calcd for $C_{49}H_{62}N_6O_8 \cdot H_2O$ (881.1): C, 66.80; H, 7.32; N, 9.54. Found: C, 66.95; H, 7.18; N, 9.51.

*4.5. 10-*tert*-Butyl-8,12-bis-(2-methoxycarbonylethyl)-2,3,7,13,17,18-hexamethyl-(21H,24H)-bilin-1,19-dione (8)*

To a solution of 10-*tert*-butyl rubin **13**[10] (64.4 mg, 0.100 mmol) in 30 ml of dry THF was added an equivalent amount (0.100 mmol, 22.7 mg) of DDQ in 10 ml of dry THF and the mixture was stirred at room temperature for 30 min. Upon addition of the DDQ 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 $CHCl_3$ and 100 ml of 2% aq. ascorbic acid then worked up as for the adamantyl ester (**7**). Removal of the solvents gave a brick-red solid. The crude product was purified by radial chromatography eluting with 100:3 (by vol) dichloromethane:methanol. This afforded 64.2 mg (80.5%) of the desired verdin (**8**) as a reddish-purple solid. It had mp of 240°C (dec.); IR (KBr) ν: 3350, 2952, 1737, 1709, 1662, 1636, 1437, 1394, 1362, 1257, 1169, 1111, 935, 757, 690 cm^{-1}; ^1H NMR ($(CD_3)_2SO$) δ: 1.40 (s, 9H), 1.74 (s, 3H), 1.80 (s, 3H), 1.92 (s, 3H), 2.05 (s, 9H), 2.20–2.47 (overlapping triplets, 8H), 3.30 (s, 3H), 3.50 (s, 3H), 5.91 (s, 1H), 6.02 (s, 1H), 9.89 (s, 1H, NH), 10.34 (s, 1H, NH), 10.37 (s, 1H, NH) ppm. ^{13}C NMR $(CD_3)_2SO$ δ: 8.51 (q), 8.60 (q), 9.56 (q), 9.61 (q), 9.67 (q), 9.75 (q), 20.72 (t), 20.78 (t), 31.99 (q), 34.21 (t), 34.24 (t), 40.58 (s), 51.12 (q), 51.53 (q), 95.58 (d), 97.19 (d), 121.02 (s), 122.27 (s), 124.47 (s), 124.81 (s), 130.13 (s), 130.28 (s), 130.78 (s), 136.41 (s), 141.39 (s), 141.81 (s), 143.60 (s), 146.66 (s), 149.89 (s), 155.41 (s), 167.09 (s), 171.54 (s), 172.21 (s), 172.36 (s), 172.76 (s) ppm. UV–vis in Table 3. Anal. calcd for $C_{37}H_{46}N_4O_6$ (642.8): C, 69.14; H, 7.21; N, 8.72. Found: C, 69.32; H, 7.36; N, 8.45.

*4.6. 10-*tert*-Butyl-8,12-bis-(2-carboxyethyl)-2,3,7,13,17,18-hexamethyl-(21H,24H)-bilin-1,19-dione (2)*

10-*tert*-Butyl verdin dimethyl ester (**8**) (27 mg, 0.04 mmol) and 20 mg of ascorbic acid were dissolved in 25 ml of 1:1 THF:MeOH (by vol). NaOH (25 ml of 0.10 M solution) was added, and the mixture was stirred at 37–40°C for 90 min. The cooled basic solution was extracted once with $CHCl_3$ to remove any remaining ester, and the aqueous solution was acidified with glacial acetic acid at 0°C. The resultant red-orange precipitate was collected by filtration, washed with water, and dried. It had low solubility in most common organic solvents and was dissolved in dichloromethane:methanol for purification by radial chromatography. Elution with 95:4:1 (by vol) dichloromethane:methanol:acetic acid afforded 21 mg (83%) of the desired diacid (**2**). It had mp of 280°C (dec.); IR (KBr) ν: 3428, 2924, 2360, 1684, 1632, 1447, 1395, 1256, 1171, 1110, 940 cm^{-1}; ^1H NMR ($(CD_3)_2SO$, 75°C) δ: 1.82 (s, 9H), 2.21 (brs, 9H), 2.44 (brs, 9H), 2.49 (m, 4H), 2.89 (m, 4H), 6.41 (brs, 2H), 10.25 (brs, 1H, NH), 10.78 (brs, 2H, NH), 12.00 (brs, 2H, CO_2H) ppm. ^{13}C NMR ($(CD_3)_2SO$) 75°C δ: 8.62 (q), 9.75 (q), 20.91 (t), 32.01 (q), 34.61 (t), 39.92 (s), 102.02 (d), 113.99 (s), 129.61 (s), 141.68 (s), 142.24 (s), 149.99 (s), 151.20 (s), 165.31 (s), 171.97 (s), 173.69 (s) ppm. UV–vis in Table 3. Anal. calcd for $C_{35}H_{42}N_4O_6$ (614.7): C, 68.38; H, 6.89; N, 9.11. Calcd for $C_{35}H_{42}N_4O_6 \cdot CH_3CO_2H$ (675.7): C, 65.70; H, 7.01; N, 8.24. Found: C, 65.52; H, 6.75; N, 8.22.

4.7. 10-tert-Butyl-8,12-bis-(2-carboxyethyl)-2,3,7,13,17,18-hexamethyl-(21H,24H)-bilin-1,19-dione bis-amide with (+)-S-alanine methyl ester (5)

10-*tert*-Butyl-rubin bis-amide[10] (56 mg, 0.091 mmol) was placed into a 100 ml round bottom flask and dissolved in 30 ml of dry THF. A solution of an equimolar amount of DDQ (21 mg) dissolved in 10 ml dry THF was added, and the mixture was stirred at room temperature for 30 min. The red solution was poured into a 2-phase system of 100 ml CH_2Cl_2 and 100 ml of 2% aqueous ascorbic acid, then worked up exactly as in the verdin dimethyl ester **8** explained earlier. The crude product was purified by radial chromatography eluting with 100:4 (by vol) dichloromethane:methanol. The red residue was dissolved in a minimum amount of CH_2Cl_2 and precipitated by the addition of hexane. This afforded 28 mg (40%) of the desired verdin (**5**) as a reddish-purple solid. It had mp 160°C (dec); UV–vis ε (λ^{max}, nm): [benzene: 37,270 (398), 7210 (505)]; [$CHCl_3$: 41,250 (410), 7000 (514)]; [CH_2Cl_2: 42,870 (407), 7850 (514)]; [THF: 41,670 (399), 6770 (522)]; [EtOH: 45,600 (406), 39,200 (382), 7660 (512)]; [DMSO: 47,430 (411), 42,230 (387), 7100 (540)]; [N-methylformamide: 48,190 (407), 44,000 (389)^{sh}, 7840 (527)]; IR (KBr) ν: 3415, 3349, 2949, 2360, 1742, 1680, 1658, 1630, 1536, 1447, 1392, 1353, 1258, 1214, 1170, 1109, 1059, 981, 936 cm^{-1}; ^1H NMR ($(CD_3)_2SO$) δ: 1.10 (d, 1.5H, J=7.3 Hz), 1.15 (d, 1.5H, J=7.3 Hz), 1.19 (d, 1.5H, J=7.3 Hz), 1.20 (d, 1.5H, J=7.3 Hz), 1.40 (s, 9H), 1.74 (s, 3H), 1.82 (s, 3H), 1.91 (s, 3H), 2.04 (s, 3H), 2.05 (s, 1.5 H), 2.06 (s, 1.5 H), 2.08 (s, 3H), 2.30 (m, 4H), 2.46 (m, 4H), 3.54 (s, 3H), 3.55 (s, 3H), 4.14 (m, 2H), 5.94 (s, 1H), 6.02 (s, 1H), 7.72 (d, 0.5H, J=6.8 Hz, CONH), 7.78 (d, 0.5H, J=7.3 Hz, CONH), 8.26 (d, 0.5H, J=6.8 Hz, CONH), 8.27 (d, 0.5H, J=6.8 Hz, CONH), 9.85 (s, 1H, NH), 10.31 (s, 0.5H, NH), 10.34 (s, 0.5H, NH), 10.38 (s, 1H, NH) ppm; ^{13}C NMR ($(CD_3)_2SO$) δ: 173.43 (s), 172.47 (s), 171.83 (s), 171.68 (s), 171.31 (s), 171.21 (s), 167.14 (s), 155.76 (s), 150.27 (s), 146.61 (s), 144.77 (s), 141.97 (s), 141.53 (s), 136.28 (s), 136.20 (s), 130.86 (s), 130.31 (s), 130.05 (s), 124.94 (s), 124.49 (s), 122.56 (s), 122.46 (s), 122.40 (s), 97.77 (d), 95.88 (d), 47.89 (q), 47.81 (q), 35.85 (t), 32.14 (d), 21.35 (t), 21.22 (t), 17.45 (q), 17.38 (q), 17.29 (q), 9.98 (q), 9.90 (q), 9.85 (q), 9.72 (q), 8.76 (q), 8.67 (q), 8.41 (q) ppm. Anal. calcd for $C_{43}H_{56}N_4O_8$ (785.0): C, 65.80; H, 7.19; N, 10.71. Calcd for $C_{43}H_{56}N_4O_8 \cdot 0.5H_2O$ (794.0): C, 65.05; H, 7.23; N, 10.52. Found: C, 65.19; H, 7.35; N, 10.56.

4.8. 9-Formyl-2,3,7-trimethyl-8-(2-carboxyethyl)dipyrrinone (15)

Triethyl orthoformate (5 ml) was added to a 25 ml round bottom flask containing dipyrrinone **14** (274 mg, 1 mmol) along with a magnetic stir bar, and the flask was sealed with a rubber septum and purged with N_2 for several minutes. Trifluoroacetic acid (~6 ml) was added and the mixture (which turned green quickly) was stirred under N_2 (balloon) at room temperature for 30 min. The reaction was quenched with H_2O, and the green precipitate was collected by filtration and washed with CH_2Cl_2. The filtrate contained the corresponding rubin and verdin among other pigments, but the precipitate was mainly the desired formyldipyrrinone (**15**). It was stirred in hot methanol containing a small amount of THF until most of the solid dissolved, filtered while hot, and allowed to cool. The resulting pure yellow solid weighed 140 mg (46%). It had mp of 290°C (dec.); IR (KBr) ν: 3335, 2910, 2856, 2365, 1705, 1698, 1688, 1680, 1671, 1556, 1447, 1393, 1345, 1267, 1164, 940 cm^{-1}; UV–vis: ε (λ^{max}) [CH_3OH: 27,000 (393) 22,300 (412)^{sh}]; [DMSO: ε (λ^{max}) 30,100 (397) 27,300 (419)^{sh}]; ^1H NMR ($(CD_3)_2SO$) δ: 1.75 (s, 3H), 2.02 (s, 6H), 2.39 (t, 2H, J=7.5 Hz), 2.87 (t, 2H, J=7.5 Hz), 5.88 (s, 1H), 9.56 (s, 1H, CHO), 10.33 (brs, 1H, NH), 11.36 (brs, 1H, NH) ppm; ^{13}C NMR ($(CD_3)_2SO$) δ: 8.62 (q), 8.91 (q), 9.83 (q), 19.37 (t), 35.22 (t), 95.45 (d), 123.01 (s), 127.32 (s), 130.79 (s), 131.31 (s), 132.67 (s), 136.33 (s), 142.16 (s), 172.87 (s), 173.85 (s), 178.23 (d) ppm. Anal. calcd for $C_{16}H_{18}N_2O_4$ (302.3): C, 63.57; H, 6.00; N, 9.27. Found: C, 63.79; H, 5.84; N, 8.83.

4.9. 8,12-Bis-(2-methoxycarbonylethyl)-2,3,7,13,17,18-hexamethyl-(21H,24H)-bilin-1,19-dione (9)

To a 100 ml round bottom flask was added 140 mg (0.464 mmol) of dipyrrinone aldehyde (**15**), and 127 mg (0.464 mmol) of (9*H*)-dipyrrinone **14**, 40 ml of CH_3OH and 1 ml of concd hydrochloric acid. The mixture was allowed to stir at room temperature for 5 h. The reaction mixture changed color from yellow to green to blue, then the solvent was removed and the blue residue was redissolved in CH_2Cl_2 and extracted first with 5% aq. $NaHCO_3$ to remove any unesterified products, and then with H_2O. The blue organic solution was dried over anhyd. Na_2SO_4 and evaporated to dryness. The crude product was purified first by radial chromatography, eluting with 4:100 CH_2Cl_2:MeOH, then by recrystallization from CH_2Cl_2:hexane to afford 103 mg (53%) of the desired verdin diester (**9**). It had mp of 250°C (dec.); IR (KBr) ν: 3449, 2953, 1736, 1686, 1630, 1592, 1439, 1240, 1158, 1093 cm^{-1}; UV–vis: ε (λ^{max}) [C_6H_6: 50000 (368), 22590 (658)]; [CH_2Cl_2: 55930 (365), 16450 (634)]; [$CHCl_3$: 53140 (367), 15050 (636)]; [$(CH_3)_2CO$: 55890 (364), 16490 (626)]; [CH_3OH: 54450 (362), 15280 (641)]; [$(CH_3)_2SO$: 5510 (371), 17760 (633)]; ^1H NMR ($(CD_3)_2SO$) δ: 1.65 (s, 6H), 2.00 (s, 12H), 2.47 (t, 4H, J=7.5 Hz), 2.83 (t, 4H, J=7.5 Hz), 3.54 (s, 6H, OCH_3), 5.92 (s, 2H), 6.87 (s, 1H), 9.87 (brs, 2H, NH) ppm; ^{13}C NMR ($(CD_3)_2SO$) δ: 8.60 (q), 9.43 (q), 9.70 (q), 19.62 (t), 35.24 (t), 51.58 (q), 96.17 (d), 127.82 (s), 128.71 (s), 137.81 (s), 140.22 (s), 141.02 (s), 141.58 (s), 149.73 (s), 172.63 (s), 172.86 (s) ppm. UV–vis in Table 3. Anal. calcd for $C_{33}H_{38}N_4O_6$ (586.7): C, 67.56; H, 6.53; N, 9.55. Found: C, 67.48; H, 6.64; N, 9.33.

4.10. 8,12-Bis-(2-carboxyethyl)-2,3,7,13,17,18-hexamethyl-1,19,21,24-tetrahydro-1,19-dioxobilin (3)

Verdin dimethyl ester (**9**) (101 mg, 0.172 mmol) and 80 mg of ascorbic acid were dissolved in 100 ml of 1:1 THF:MeOH (by vol). Next, 100 ml of 0.10 M NaOH was added, and the mixture was stirred at 37–40°C for 90 min. The cooled basic solution was extracted once with $CHCl_3$ to remove any remaining ester, and the aqueous solution was acidified with glacial acetic acid at 0°C. The resultant blue precipitate was collected by filtration, washed with water, and dried. It had low solubility in most common organic solvents but was dissolved in dichloromethane:methanol for purification by radial chromatography. Elution with 100:6 (by vol) dichloromethane:methanol afforded 22 mg (22%) of the desired blue verdin, after precipitation from dichloromethane:methanol:hexane. It had mp of 325°C (dec.); IR (KBr) ν: 3412, 2997, 2910, 2823, 1660, 1436, 1404, 1311, 1033, 951, 897, 700 cm^{-1}; ^1H NMR ($(CD_3)_2SO$) δ: 1.65 (s, 6H), 2.01 (s, 12H), 2.34 (t, 4H, J=7.5 Hz), 2.79 (t, 4H, J=7.5 Hz), 5.91 (s, 2H), 6.98 (s, 1H), 9.88 (brs, 2H, NH), 11.98 (brs, 2H, CO_2H) ppm. ^{13}C NMR ($(CD_3)_2SO$) δ: 8.60 (q), 9.43 (q), 9.70 (q), 19.62 (t), 35.24 (t), 96.17 (d), 115.99 (d), 127.82 (s), 128.71 (s), 137.81 (s), 140.22 (s), 141.02 (s), 141.58 (s), 146.51 (s), 149.73 (s), 172.63 (s), 172.86 (s) ppm. UV–vis in Table 3. Anal. calcd for $C_{31}H_{34}N_4O_6$ (558.6): C, 66.65; H, 6.13; N, 10.03. Found: C, 66.23; H, 6.51; N 10.03.

4.11. 8,12-Bis-(2-carboxyethyl)-2,3,7,13,17,18-hexamethyl-1,19,21,24-tetrahydro-1,19-dioxobilin bis-amide with S-(+)-alanine methyl ester (6)

Verdin **3** (21.3 mg, 0.038 mmol), *S*-(+)-alanine methyl ester hydrochloride (53.3 mg, 0.382 mmol), diphenylphosphoryl azide (DPPA) (83 μl, 0.38 mmol), and triethylamine (106 μl, 0.764 mmol) were mixed in 2 ml of dry DMSO at room temperature and stirred in the dark for 24 h. To the cloudy solution was added 100 ml of CH_2Cl_2, and the solution was washed with water (4×50 ml), then with satd aq. NaCl solution and dried over anhydrous Na_2SO_4. Removal of the solvents left a blue residue which was purified by radial chromatography eluting with 100:3 (by vol.) dichloromethane:methanol. The blue film was dissolved in hot CH_2Cl_2 and precipitated by slow addition of hexane with swirling to afford 10.8 mg

(39%) of the desired verdin (**6**). It had mp of 240°C (dec.); IR (KBr) ν: 3433, 3063, 2943, 2910, 1741, 1687, 1660, 1589, 1540, 1453, 1387, 1273, 1213, 1153, 1088, 1055, 962, 891, 821, 755, 690 cm^{-1}; UV–vis: ε (λ^{max}) [C_6H_6: 53150 (367), 17600 (627)]; [CH_2Cl_2: 56600 (366), 16280 (627)]; [$CHCl_3$: 56970 (367), 16650 (630)]; [CH_3CH_2OH: 55450 (365), 15630 (639)]; [THF: 52150 (367), 17060 (622)]; [$(CH_3)_2SO$: 57100 (371), 18070 (632)]; ^1H NMR ($(CD_3)_2SO$) δ: 1.18 (d, 6H, J=6.8 Hz), 1.65 (s, 6H), 2.01 (s, 12H), 2.27 (t, 4H, J=7.8 Hz), 2.75 (t, 4H, J=7.3 Hz), 3.55 (s, 6H), 4.20 (q, 2H, J=7.3 Hz), 5.93 (s, 2H), 6.82 (s, 1H), 7.24 (d, 2H, J=6.8 Hz, CONH), 9.87 (brs, 2H, NH) ppm; ^{13}C NMR ($(CD_3)_2SO$) δ: 8.65 (q), 9.39 (q), 9.67 (q), 19.56 (t), 32.49 (d), 34.33 (t), 47.90 (q), 97.00 (d), 16.03 (d), 127.64 (s), 129.39 (s), 136.98 (s), 139.87 (s), 141.09 (s), 141.67 (s), 146.33 (s), 150.11 (s), 172.67 (s), 172.92 (s) ppm. Anal. calcd for $C_{39}H_{48}N_6O_8$ (728.8): C, 64.27; H, 6.64; N, 11.53. Calcd for $C_{39}H_{48}N_6O_8 \cdot 0.5H_2O$ (737.8): C, 63.48; H, 6.83; N, 11.39. Found: C, 63.62; H, 6.63; N, 11.34.

4.12. Molecular dynamics

Molecular mechanics calculations and molecular modeling were carried out on an Evans and Sutherland ESV-10 workstation using version 6.0 of SYBYL (Tripos Assoc., St. Louis, MO). The dipyrrinone unit of **1–3** was rotated about the central CHR C(9)–C(10) bond through 10° increments from 0° to 360°. In this procedure the torsion angle was held fixed at each increment while the remainder, especially torsions about the C(5)–C(6) and C(14)–C(15) bonds, of the molecule was relaxed to its minimum energy conformation using molecular mechanics. This was followed by a molecular dynamics cooling curve consisting of the following temperatures and times: 100 fs at 20 K, 100 fs at 10 K, 100 fs at 5 K, 200 fs at 2 K, 200 fs at 1 K, 200 fs at 0.5 K, 300 fs at 0.1 K. This was followed by molecular mechanics minimization, which gave the lowest energy conformations for each ϕ value. The ball and stick drawings were created from the atomic coordinates of the molecular dynamics structures using Müller and Falk's 'Ball and Stick' program (Cherwell Scientific, Oxford, UK) for the Macintosh.

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