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SYNTHESIS AND CONFORMATION OF A BILIRUBIN ANALOG WITH PROPIONIC ACID SIDE CHAINS EXTENDED TO UNDECANOIC ACID

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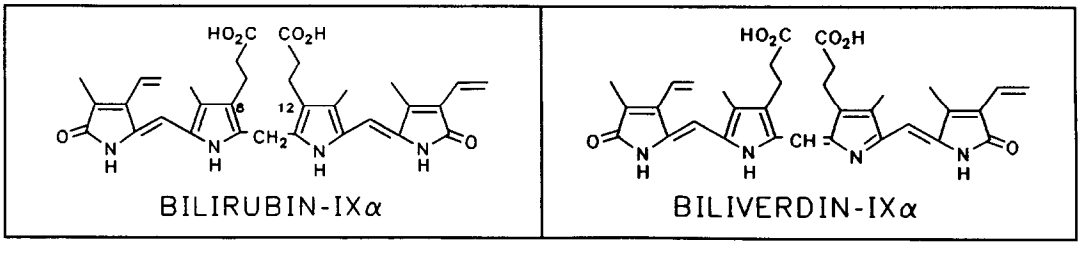
Abstract. **A lipophilic analog of bilirubin with propionic acid groups replaced by undecanoic acid groups (1), was synthesized from 11-bromo-undecanoic acid. UV-visible and NMR spectroscopic analyses suggest reduced intramolecular H-bonding and a preference for a helical conformation. Molecular dynamics computations predict a global energy minimum for a helical porphyrin-like conformation with unusual distorted structures when H-bonding is invoked. Circular dichroism of the pigment complex with human serum albumin gives a bilirubin-like bisignate Cotton effect: $\Delta\epsilon_{449}^{max} = +33$, $\Delta\epsilon_{396}^{max} = -47$.**

INTRODUCTION

Complicated, structurally interesting linear tetrapyrroles such as the natural products bilirubin and biliverdin are formed in animal metabolism from normal turnover of hemoglobin and other heme proteins.[1-3] Considerable effort has been devoted to understanding the properties and metabolism of bilirubin, with particular attention being focussed on its unique ability to fold into a conformation where the carboxylic acid groups are sequestered through intramolecular hydrogen bonding.[4-6] That lowers their acidity, decreases the polarity of the pigment and renders it unexcretable in normal metabolism, except by glucuronidation. Recently it has become evident that translocation of the pigment's propionic acid groups away from the natural locations at C-8 and C-12 leads to pigments which were more polar than bilirubin and do not require glucuronidation for hepatic excretion.[7,8] However, bilirubin analogs with propionic acid groups at C-8 and C-12, *e.g.*, mesobilirubin-XIIIα, typically exhibit the same unique polarity and excretability properties as bilirubin. For these pigments, like bilirubin, can tuck their carboxylic acid groups inward, where they are tethered to an opposing dipyrrinone by intramolecular hydrogen bonding (see Figure 1).



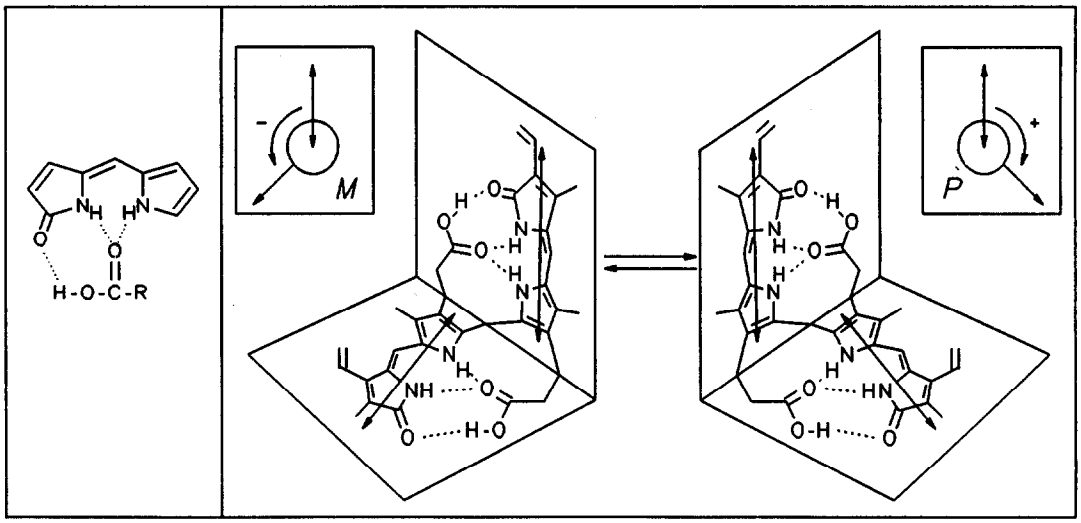
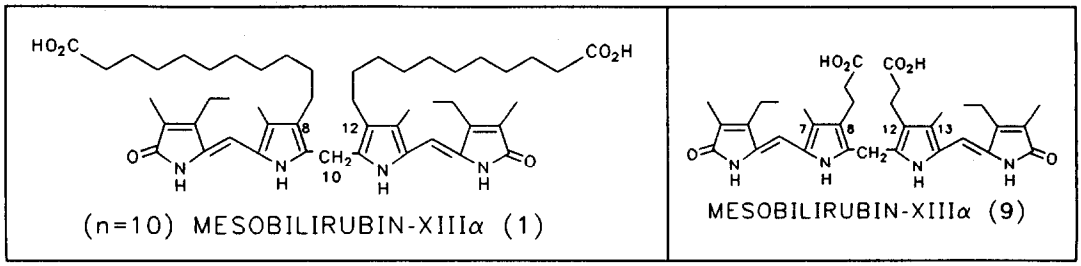
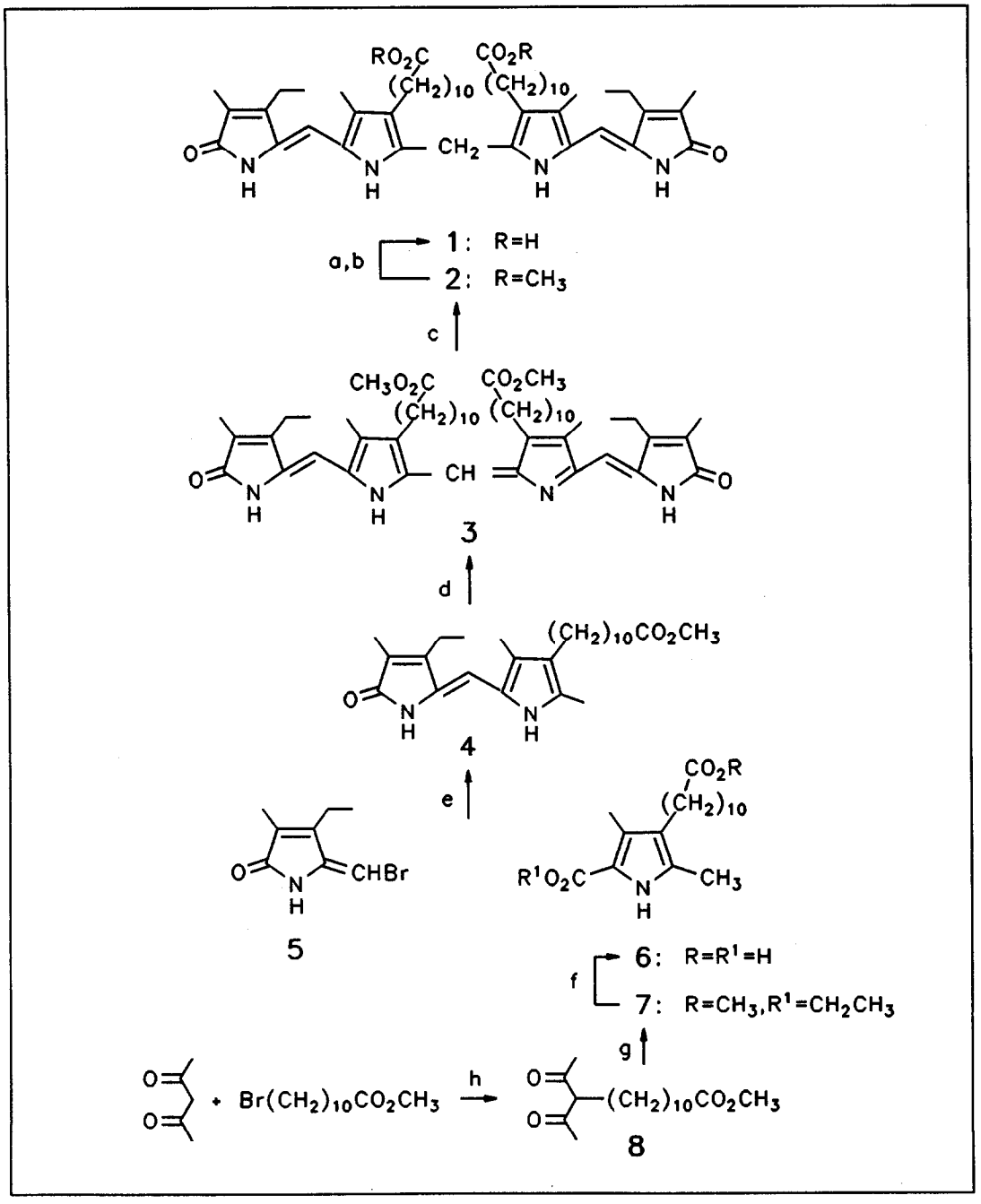


FIGURE 1. (Left) Partial structure showing a dipyrrinone fragment hydrogen bonded to a carboxylic acid group. (Right) Interconverting intramolecularly hydrogen-bonded enantiomeric conformers of bilirubin-IXα. The double headed arrows represent the dipyrrinone long wavelength electric transition moment vectors (dipoles). The relative helicities (*M*, minus or *P*, plus) of the vectors are shown (inset) for each enantiomer.

Such intramolecular hydrogen bonding, depicted in Figure 1, is one of the most interesting and important facets of bilirubin structure.[1,4-7,9] Although the two component dipyrrinone units of bilirubin-type molecules may rotate relatively freely and independently about the interconnecting central -CH_2- group, two conformations are uniquely stabilized through an extensive network of intramolecular hydrogen bonds. These two conformations are non-superimposable mirror images, which (for bilirubin) are known to interconvert fairly rapidly at room temperature over a barrier of ~20 kcal/mole.[5,6] Our interest in pigment stereochemistry that is stabilized by intramolecular hydrogen bonding between propionic acid and dipyrrinone groups led us to consider: (1) whether such hydrogen bonding might be retained in a bilirubin analog where both propionic acid groups are replaced by longer alkanoic acid groups, and (2) how such hydrogen bonding might affect the conformation of the pigment. In the following, we report on the synthesis, properties and conformational analysis of a new, symmetric analog (**1**) of mesobilirubin-XIIIα (**9**), with undecanoic acid groups replacing the conventional propionic acids. The spectral properties of (**1**), which we call (n=10) mesobilirubin-XIIIα, are correlated with a stereochemical analysis from molecular dynamics simulations using SYBYL on an Evans and Sutherland ESV-10 workstation.



SYNTHETIC SCHEME



[a]NaOH/THF; [b]H_3O^+; [c]$NaBH_4$; [d]chloranil/HCO_2H/reflux; [e]CH_3OH/reflux; [f]NaOH/$NaNO_3$/Δ, then HNO_3/$NaNO_3$; [g]$CH_3COC(NOH)CO_2CH_2CH_3$/Zn/HO_2CCH_3; [h]K_2CO_3-Cs_2CO_3/CH_3CN-$(CH_3)_2SO$/65°.

RESULTS AND DISCUSSION

Synthesis.

The target bilirubin, (n=10) mesobilirubin-XIIIα (**1**), was reached through the series of steps outlined in the Synthetic Scheme. Thus, alkylation of pentane-2,4-dione by methyl 11-bromo-undecanoate gave the desired precursor (**8**) in 63% yield according to the literature method.[10] The ratio of C-alkylation to O-alkylation was 7:3, comparable to that (72:28) seen when ethyl 6-bromohexanoate was used in the alkylation.[10] Initial attempts to use the unseparated O- and C-alkylated mixture in the pyrrole synthesis gave a difficultly separable mixture; so, the O-alkylated pentane-2,4-dione was removed by refluxing in glacial acetic acid in the presence of sodium acetate. The resulting pure C-alkylated material (**8**) was treated in a modified Fischer-Knorr pyrrole synthesis to afford a 64% yield of **7**.[11] The modifications included using a two-fold excess of nitrosated ethyl acetoacetate formed from sodium nitrite in a minimal amount of water, and introduction of **8** in tetrahydrofuran. Conversion of **7** to **6** was achieved by saponification, and coupling with the known bromomethylene-oxopyrrole (**5**) to give dipyrrinone **4** was achieved in refluxing methanol in an unoptimized 36% yield. Oxidative coupling of dipyrrinone **4** using *p*-chloranil in hot 98% formic acid[12] gave (n=10) verdin **3** in 47% yield as dark blue needles. Reduction of **3** to the (n=10) mesobilirubin dimethyl ester (**2**) proceeded smoothly in (10:1 v/v) 2-propanol/methanol solvent, and saponification to the diacid (**1**) was achieved by treating **2** with a deaerated dilute solution of NaOH in methanol-tetrahydrofuran at reflux. It is important to exclude oxygen at this step; otherwise, the reaction mixture rapidly turns green and purification of **1** becomes difficult.

Polarity from Chromatographic Behavior.

The (n=10) mesobilirubin-XIIIα (**1**) has a longer retention time (~19.7 minutes) when coinjected with the parent (n=2) mesobilirubin-XIIIα (**9**) with propionic acid groups at C-8 and C-10 (~11.4 minutes) on reverse phase HPLC, suggesting that **1** is more lipophilic than the parent. However, on silica gel TLC, **1** has a much smaller R_f value (0.0) when compared with the (n=2) mesobilirubin-XIIIα standard (R_f=0.87) using CH_2Cl_2:CH_3OH (9:1, vol/vol) as eluant, suggesting that it is much more polar than **9**.

TABLE 1. Comparison of Mesobilirubin Lactam and Pyrrole N-*H* Chemical Shifts[a] in $CDCl_3$ and $(CD_3)_2SO$ Solvents.[b]

Pigment	$CDCl_3$			$(CD_3)_2SO$	
	Lactam	Pyrrole	CO_2H	Lactam	Pyrrole
1	10.53	9.20	13.56	9.79	10.23
Mesobilirubin-XIIIα (**9**)	10.57	9.15	13.62	9.72	10.27
Etiobilirubin-IVγ	10.58	10.28	—	9.78	10.28
Mesobilirubin-XIIIα Dimethyl Ester	10.54	10.27	—	9.74	10.40
2	10.63	10.30	—	9.72	10.38

[a] δ, ppm downfield from $(CH_3)_4Si$; [b] Run as 10^{-2} *M* $(CD_3)_2SO$ and 10^{-3} *M* $CDCl_3$ solutions at 22°C.

NMR Analysis and Intramolecular Hydrogen Bonding.

The ^{1}H-NMR N-*H* chemical shifts of the pyrrole and lactam have proven to be an excellent way to determine whether the dipyrrinone units of bilirubins are involved in intramolecular hydrogen bonding.[12-14] Previous studies of bilirubin pigments have shown that the pyrrole N-*H* appears near 9.2 δ in $CDCl_3$ solvent (*e.g.* for **9**) when the dipyrrinone and carboxylic acid groups are intramolecularly hydrogen bonded, as shown in Figure 1.[13,14] When **9** is esterified, however, or when its propionic acid groups are relocated to C-7 and C-13 (as in mesobilirubin-IVα), or when they are replaced by ethyl (as in etiobilirubin-IVγ), the pyrrole hydrogens become more deshielded (10.3 δ) due to dipyrrinone-dipyrrinone intermolecular hydrogen bonding (Table 1).[15,16] In $(CD_3)_2SO$, all of the dipyrrinone N-*H*s become hydrogen bonded to solvent; so, the distinctions due to self-association and intramolecular hydrogen bonding seen in $CDCl_3$ are lost, and all pyrrole N-*H* resonances appear near 10.4 δ. It was anticipated that the pyrrole (and lactam) N-*H* chemical shifts of **1** and **9** would be the same in $(CD_3)_2SO$ solvent (Table 1). The data also reveal a characteristic pyrrole N-*H* chemical shift for intramolecular hydrogen bonding in $CDCl_3$. In fact, the data in $CDCl_3$ solvent for **1** and **9** are so similar that one is tempted to conclude that the CO_2H groups of **1** are tethered to the dipyrrinones as they are for **9** (Figure 1). In contrast, the dimethyl ester (**2**) of **1** exhibits N-*H* chemical shifts in $CDCl_3$ solvent expected for intermolecular dipyrrinone-dipyrrinone hydrogen bonding, as has been documented for bilirubin and mesobilirubin dimethyl ester and similar analogs.[12-15]

UV-Visible Spectral Analysis and Conformation from Exciton Coupling.

Further evidence on intramolecular hydrogen bonding comes from solvent-dependent UV-visible spectra. Over a wide range of solvents with varying polarity and hydrogen bonding ability (benzene, chloroform, methanol and dimethylsulfoxide), the UV-visible spectra of mesobilirubin-XIIIα (**9**) change very little, with λ^{max} being near 430 nm and λ^{sh} near 395 nm[12,16] — corresponding to the two exciton components from electric transition dipole-dipole interaction of the two proximal dipyrrinone chromophores approximately 90° apart (as in Figure 1).[17-19] Since mesobilirubin-XIIIα is known from NMR studies to adopt the intramolecularly hydrogen bonded conformation of Figure 1 in $CDCl_3$ solvent and a similar conformation in $(CD_3)_2SO$ solvent,[5,20] it might be argued that a UV-visible exciton couplet with $\lambda^{max} \simeq$ 430 nm, $\lambda^{sh} \simeq$ 395 nm can be taken as an indicator of a folded (but not necessarily hydrogen-bonded) conformation akin to that of Figure 1. The UV-visible spectra of **1** (Table 2) are very much like those of mesobilirubin-XIIIα, especially (as might be expected) in $(CH_3)_2SO$ and CH_3OH solvents. They differ somewhat in nonpolar solvents. For example, in $CHCl_3$, λ^{max} is now at 395 nm, with λ^{sh} at 430 nm, whereas, the reverse is the case for mesobilirubin-XIIIα. The data for **1** are consistent with a folded conformation, probably one involving intramolecular hydrogen bonding in $CHCl_3$ and other non-polar solvents, but with the conformation deformed to a smaller dihedral angle[18] giving the pigment a helical shape.

The UV-visible spectral solvent dependence of dimethyl ester **2** is different from that of **1** (Table 2) but quite similar to that of mesobilirubin-XIIIα dimethyl ester, which typically exhibits a strong solvent, concentration and temperature dependence due to formation of dimers[21] in non-polar solvents such as benzene and chloroform. This results in spectra with a narrow bandwidth intense absorption at λ^{max} near 380 nm and weak shoulder at λ^{sh} near 430 nm.[12,16] In more polar solvents such as CH_3OH and $(CH_3)_2SO$ the solutions are largely monomeric, and the UV-visible spectra are thus quite similar to those of the parent acid in these solvents, with λ^{max} near 435 nm and λ^{sh} near 400 nm.[12,14a] Corroborating evidence for

folded structures in $(CD_3)_2SO$ and intermolecularly hydrogen bonded structures in $CDCl_3$ comes from NMR studies,[5,13] and the intermolecularly hydrogen bonded pigments are probably constrained to adopt folded structures with smaller dipyrrinone-dipyrrinone (interplanar) dihedral angles.[5a,13] The UV-visible spectra of **2** are thus entirely consistent with the expectations drawn from earlier studies of mesobilirubin and bilirubin dimethyl esters.[13] On the other hand, the spectra of **2** are quite different from those of **1** in non-polar solvents where either intra or intermolecular hydrogen bonding can play a role. If intermolecular hydrogen bonding predominates for **2** in these solvents, then intramolecular hydrogen bonding would appear to be the important distinguishing feature in **1**.

TABLE 2. UV-Visible Spectroscopic Data for (n=10) Mesobilirubin-XIIIα (**1**) and Its Dimethyl Ester (**2**).[a]

Solvent	1				2			
	λ^{max}	ϵ^{max}	λ^{sh}	ϵ^{sh}	λ^{max}	ϵ^{max}	λ^{sh}	ϵ^{sh}
Hexane	390	29,400	440	27,100	377	79,000	430	14,200
Benzene	394	43,600	430	31,100	381	76,000	430	17,900
CH_2Cl_2	394	44,400	428	32,200	379	77,600	430	15,200
$CHCl_3$	395	47,900	430	33,500	380	72,600	430	19,900
Ethyl acetate	382	51,700	419	31,000	377	76,600	425	19,700
CH_3CN	379	53,800	430	21,500	377	75,900	425	17,400
C_2H_5OH	438	56,500	395	42,400	440	67,500	400	43,900
CH_3OH	437	59,500	405	44,600	438	68,900	400	46,200
$(CH_3)_2SO$	434	61,600	390	40,000	436	71,500	400	45,800

[a] Run at 1-2 x 10^{-5} *M* concentrations; λ^{max} and λ^{sh} in nm, ϵ^{max} and ϵ^{sh} in M^{-1} cm^{-1}.

Conformational Analysis from Molecular Dynamics Calculations.

Additional insight may be gained from an analysis of conformation by molecular dynamics methods using the force field in SYBYL. A conformational energy map (Figure 2) can be constructed by rotating the dipyrrinone units independently about the 9-10 and 10-11 carbon-carbon single bonds, corresponding to torsion angles ϕ_1 and ϕ_2, respectively. With ϕ_1 and ϕ_2 defined as 0° in the porphyrin-like conformation (Figure 3), a large array of conformations can be created through rotations about ϕ_1 and ϕ_2, e.g., the linear conformation, with $\phi_1 = \phi_2 = 180°$. Figure 2 shows a mapping of conformational energy *vs* rotation angles, ϕ_1 and ϕ_2. Some conformations are stabilized through intramolecular hydrogen bonding.[18,22,23] In the absence of intramolecular hydrogen bonding, the global energy minimum conformation of **1** is essentially the same as that of **9** and other bilirubin pigments — a ridge-tile shape.[18,23] In **9** intramolecular hydrogen bonding stabilizes the ridge tile conformation. In **1**, however, intramolecular hydrogen bonding is also conformation stabilizing, but the much longer alkanoic acid chain dictates a new stable conformation at the global minimum. Consequently, the potential energy surface of **1** (Figure 2) is very different in appearance

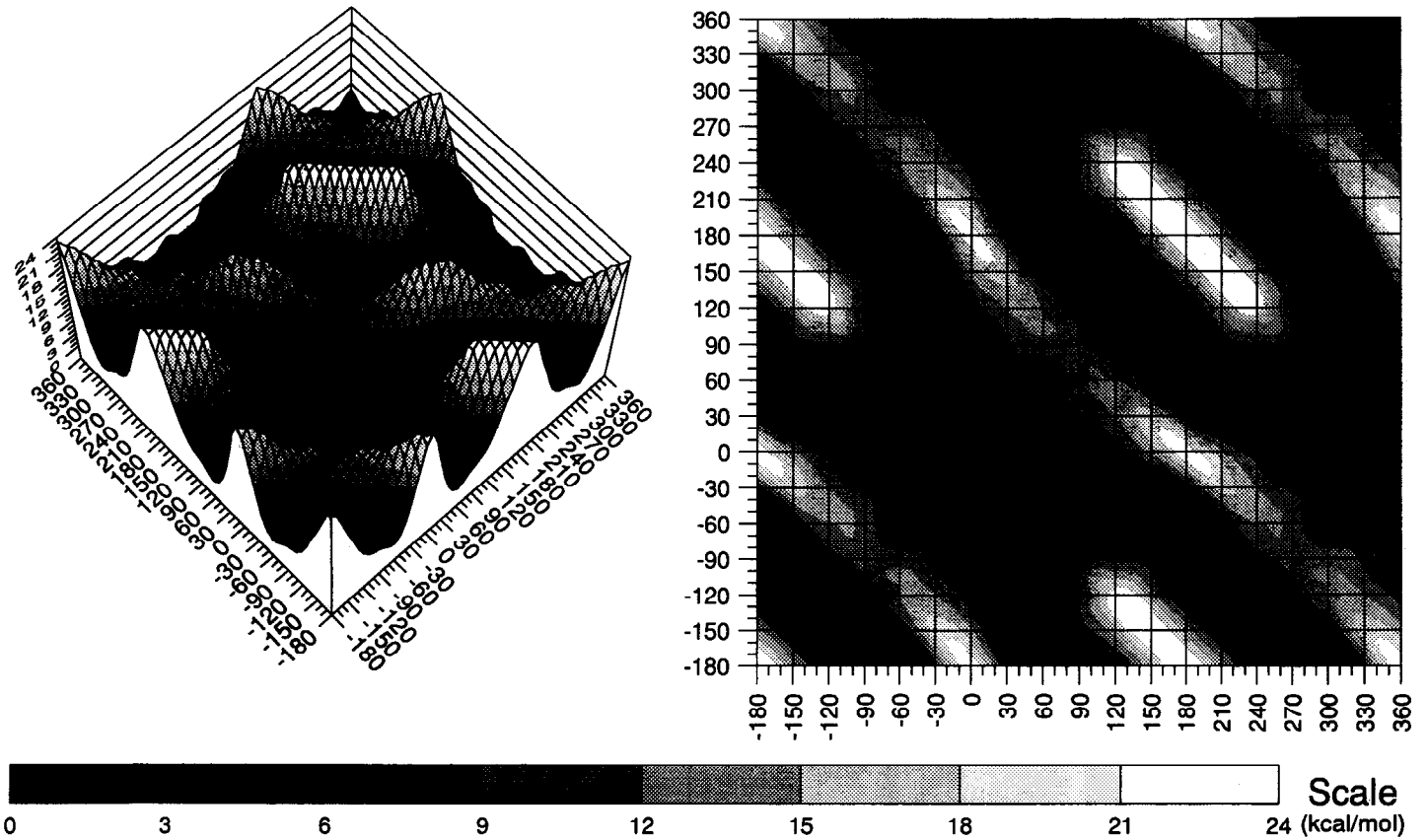


FIGURE 2. Potential energy surface (Left) and contour maps (Right) for (n=10) mesobilirubin-XIIIα conformations generated by rotating the two dipyrrinone groups independently about the C9-C10 and C10-C11 bonds (ϕ_1 and ϕ_2 respectively). The energy scale (below) is in kcal/mol, and the global minima (set to 0 kcal/mol) for pairs of mirror image intramolecularly hydrogen bonded conformations are found near (ϕ_1, ϕ_2) = (-70°, 50°) and (70°, -50°), (-50°, 70°) and (50°, -70°); or (70°, 310°) and (290°, 50°), (50°, 290°) and (310°, 70°). Conformations with (ϕ_1, ϕ_2) = (-70°, 50°), (50°, -70°), (290°, 50°) and (50°, 290°) are identical, as are the following conformations (70°, -50°), (-50°, 70°), (70°, 310°) and (310°, 70°). Local minima are located near (ϕ_1, ϕ_2) = (60°, 180°), (180°, 60°), (180°, 300°), (300°, 180°) etc... and lie ~1.3 kcal/mol above the global minima. Data are from molecular dynamics simulations using SYBYL® (Tripos assoc.) on an Evans & Sutherland ESV-10 workstation. The energy surface display was created using Wingz™ (Informix).

from that of **9**.[18,22,23] The longer acid (n=10) chains force the molecule to adopt a different set of global minima than the natural (n=2) propionic acid chains of **9**. The global minima correspond to identical or enantiomeric structures of **1**, with each enantiomer being represented by several different points on the surface of Figure 2. Thus global minima are found for identical **M**-helicity conformers (Figure 4) near (ϕ_1,ϕ_2) = (50°,290°), (290°,50°), (50°,-70°) and (-70°,50°) and for isoenergetic **P**-helicity conformers located near (ϕ_1,ϕ_2) = (70°,310°), (310°,70°), (70°,-50°) and (-50°,70°). Through the action of intramolecular hydrogen bonding, the molecule adopts one basic three-dimensional molecular structure corresponding to the cited minima, a conformation where the two dipyrrinone chromophores are aligned in a skewed porphyrin-like conformation with one acid chain wrapped around each side of the resulting trough-shaped structure. This conformation represents a departure from the typical ridge-tile[4a] conformation[18] corresponding to global minima ($\phi_1=\phi_2\simeq 60°$) found for bilirubin and **9**, which have shorter (propionic) acid chains. In intramolecularly hydrogen-bonded **1** the long side chains push the erstwhile ridge-tile conformation into a more closed shape with a lower energy. The global minima of **1** lie about 10 kcal/mole below the ridge-tile conformation. Secondary minima are located near (ϕ_1,ϕ_2) = (60°, 180°), (180°,60°), etc. and their enantiomeric counterparts at (180°,300°), (300°,180°), etc. and lie ≈1.3 kcal/mol above the global minima. These conformations also use the same hydrogen bonding scheme as is seen in **9**, but the pigment backbone geometry is skewed and somewhat extended to compensate for the longer chains. These are conformers where the central torsion angles are increased beyond the range of the ridge-tile conformation, past (ϕ_1,ϕ_2) = (130°,130°), and they, too, must therefore distort in order to maintain the intramolecular hydrogen bonding.

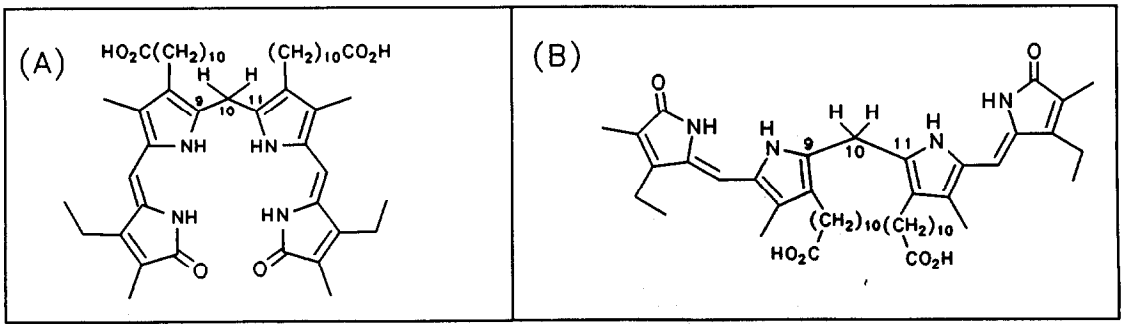


FIGURE 3. (n=10) Mesobilirubin-XIIIα (**1**) in the porphyrin-like conformation (A), and the linear conformation (B). The representations (A) and (B) may be interconverted by rotating the dipyrrinones about the 9-10 and 10-11 bonds (torsion angles ϕ_1 and ϕ_2). The porphyrin-like conformation (A) corresponds to $\phi_1=\phi_2=0°$, and the linear or extended conformation (B) corresponds to $\phi_1=\phi_2=180°$. There are a multitude of conformations lying between these two extremes, and some are stabilized by intramolecular hydrogen bonds between the undecanoic acid CO_2H and the dipyrrinones, *viz* those shown in Figure 4 with $\phi_1=50°$, $\phi_2=290°$ (or its mirror image at $\phi_1=310°$, $\phi_2=70°$) and $\phi_1=60°$, $\phi_2=180°$ (or its mirror image at $\phi_1=300°$, $\phi_2=180°$).

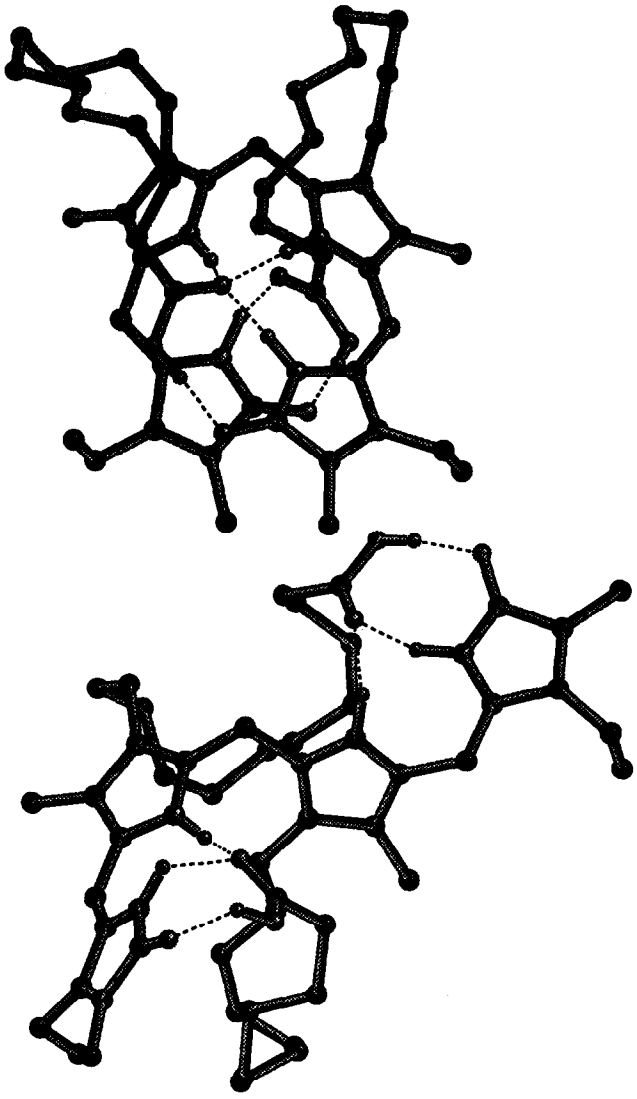


Figure 4. (Upper) Global minimum energy conformation of intramolecularly hydrogen bonded (n=10) mesobilirubin-XIIIα (**1**) at $\phi_1,\phi_2 = 50°,290°$ corresponding to the M-helicity. (Lower) Local minimum energy conformation **1** located at $\phi_1,\phi_2 = 60°,180°$ and lying ~1.3 kcal/mol higher in energy than the global minimum. (Hydrogens on carbons are removed for clarity of presentation, hydrogen bonds between CO_2H and dipyrrinone pyrrole NH and lactam -NH-C=O groups are represented by dashed lines.)

Circular Dichroism and Binding to Albumin.

As may be seen in Figure 5, a solution of **1** in buffered solution with 2 mole equivalents of human serum albumin (HSA) gives a well-defined, intensely bisignate circular dichroism (CD) spectrum for the long wavelength UV-visible transition. The CD is similar in sign and magnitude to that observed for bilirubin-IXα. When bound to HSA and other species' serum albumin, bilirubin-IXα is known to exhibit optical activity, seen typically as an induced circular dichroism (CD), which is usually intense and bisignate.[24,25] The origin of the optical activity comes from the pigment adopting a chiral conformation selected at the binding site on the protein. The observed bisignate CD comes from exciton coupling of two electric dipole transitions (Figure 1): one from each of the pigment's twin dipyrrinone chromophores, *viz.* those from the long wavelength UV-visible excitation near 421 nm. The protein is acting as an enantioselective binding agent and constrains the pigment to adopt a chiral conformation, but as reported previously,[24] the presence of at least one propionic acid group at C-8 or C-12 is essential to the enantioselectivity in binding. Thus, bilirubin pigments with both propionic acid groups esterified as methyl esters give only very weak induced CDs, as do analogs with no acid groups, e.g. etiobilirubin-IVγ. As expected, therefore, diester **2** gave only a negligible CD (Table 3). In contrast, **1**, the bilirubin analog with undecanoic acid rather than propionic acid chains at C-8 and C-12, gives an intense bisignate CD. This finding reinforces the notion that CO_2H groups are essential to the enantioselectivity by the protein, even when they are at the terminus of a long fatty acid chain. What is surprising is that the long acid chain length and lipid character of the pigment would appear to have little influence on the enantioselection, for the CD signs and magnitudes observed are nearly the same as those observed for bilirubin itself (Table 3).

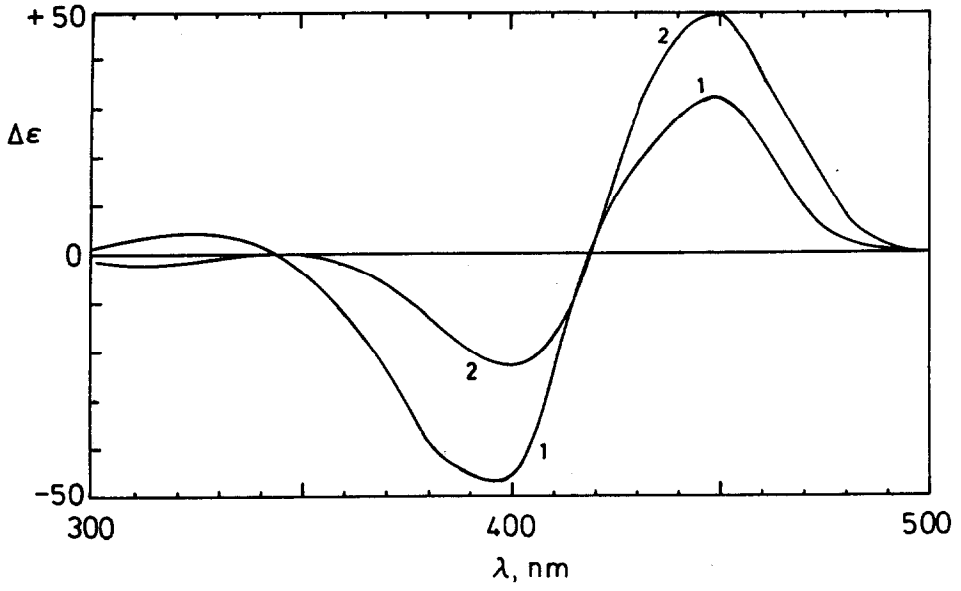


FIGURE 5. Comparison of the circular dichroism (CD) spectra of (n=10) mesobilirubin-XIIIα (curve 1) on human serum albumin (HSA) with bilirubin-IXα on HSA (curve 2) in pH 7.4 Tris buffer at 22°C. The concentration of pigment in each spectrum is 2×10^{-4} *M* and that of HSA is 4×10^{-4} *M*, for a 1:2 molar ratio of pigment to albumin.

TABLE 3. Comparison of Circular Dichroism and UV-visible Spectral Data[a] for (n=10) Mesobilirubin-XIIIα, Bilirubin and Etiobilirubin-IVγ in pH 7.4 Buffered Aqueous Human Serum Albumin Solutions Containing 30% Dimethylsulfoxide.[b]

Pigment	Circular Dichroism			UV-Visible
	$\Delta\epsilon_{max}$ (λ_1)	λ at $\Delta\epsilon$=0	$\Delta\epsilon_{max}$ (λ_2)	ϵ_{max} (λ)
(n=10) Mesobilirubin-XIIIα (1)	+33 (449)[c]	418	-47 (396)[c]	41,900 (450) 29,000[sh] (389)
Bilirubin-IXα	+50 (449)	425	-24 (400)	44,000 (452)
Etiobilirubin-IVγ	+2.1 (438)	414	-3.5 (388)	22,600[sh] (432) 37,300 (375)
(n=10) Mesobilirubin-XIIIα Dimethyl Ester	-0.42 (459)[d]	420	+0.06 (382)	37,100[sh] (457) 44,300 (434)

[a]$\Delta\epsilon$ and ϵ in L · mole^{-1} · cm^{-1} and λ in nm; [b]For 2-3 x 10^{-5} *M* pigment solutions run with 2 mole equivalents of human serum albumin, 30% $(CH_3)_2SO$ has no effect on the BR-HSA CD spectrum; [c]Shoulders at 431 nm ($\Delta\epsilon$ = +19) and 387 nm ($\Delta\epsilon$ = -44); [d]Shoulder at 442 nm ($\Delta\epsilon$ = -0.36).

Metabolism.

Interestingly, when **1** was administered to a Sprague-Dawley rat, neither **1** nor its glucuronide could be detected in bile. This contrasts markedly with the behavior of the parent, mesobilirubin-XIIIα, which is excreted promptly into bile, but only as mono and diglucuronides. Surprisingly, two hours after administration of **1**, the serum showed complete absence of **1** and no presence of its conjugated derivatives. Since no conjugates of **1** had appeared in bile during this time, these results may be explained by an ineffective glucuronidation and trapping of **1** in (plasma) membranes. And since the preferred substrate for bilirubin glucuronyl transferase is the partial structure in Figure 1 (or the corresponding anion), it might be concluded that **1** is not intramolecularly hydrogen bonded since it is not glucuronidated. However, glucuronidation requires hepatic uptake, which although thought not to be affected much by intramolecular hydrogen bonding, might be effectively thwarted in **1** with its long chain fatty acid groups. Alternatively, effective glucuronidation may depend on the shape of the pigment as well as its ability to hydrogen bond intramolecularly, and **1** (Figure 4) has a very different shape from that of bilirubin (Figure 1).

CONCLUDING COMMENTS

Intramolecular hydrogen bonding between propionic acid CO_2H and dipyrrinone groups is known to be a dominant, conformation stabilizing force in bilirubin and its analogs.[18] The current study shows that even when the propionic acid chains are expanded to undecanoic acid, intramolecular hydrogen bonding persists in non-polar solvents. And the effect of such hydrogen bonding is to stabilize a new conformation where the dipyrrinones have rotated out of the typical ridge-tile folded bilirubin conformation (Figure 1) and into a helical porphyrin-like conformation (Figure 4). The apparent failure of **1** to undergo glucuronidation *in vivo* may be a consequence of the new pigment geometry.

EXPERIMENTAL

General Procedures. All ultraviolet-visible spectra were recorded on a Perkin Elmer Model 3840 diode array or Cary 219 spectrophotometer, and all circular dichroism (CD) spectra were recorded on a Jasco J-600 instrument. Nuclear magnetic resonance (NMR) spectra were determined on a GE QE-300 300-MHz spectrometer in $CDCl_3$ solvent (unless otherwise specified) and reported in δ ppm downfield from $(CH_3)_4Si$. Melting points were determined 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 layer). 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 $CaSO_4$ preparative thin 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 μm C-18 ODS column (25 x 0.46 cm) and a Beckman ODS precolumn (4.5 x 0.46 cm). The flow rate was 1.0 mL/minute, and the elution solvent was 0.1 *M* di-*n*-octylamine acetate in 3% aqueous methanol (pH 7.7, 31°C).

Spectral data were obtained in spectral grade solvents (Aldrich or Fisher). Pentane-2,4-dione, ethyl acetoacetate, 11-bromo-undecanoic acid, tetrachloro-1,4-benzoquinone (*p*-chloranil), acetic acid, tetrahydrofuran, 98% formic acid, acetonitrile, dimethylsulfoxide and sodium borohydrided, were from Aldrich. Tetrahydrofuran was dried by distillation from sodium; methanol was dried (Mg, reflux) and distilled. Solutions of (n=10) mesobilirubin-XIIIα (**1**), its dimethyl ester (**2**), bilirubin-IXα and etiobilirubin-IVγ in pH 7.4 aqueous human serum albumin were prepared as reported earlier,[21] except the weighed pigment and albumin were mixed together in 3 mL of dimethylsulfoxide, cooled in ice, then diluted with Tris buffer to a final volume of 10 mL.

Methyl 12-Acetyl-13-oxotetradecanoate (8). A mixture of 11-bromo-undecanoic acid (50 g, 0.189 mol), *p*-toluenesulfonic acid (*ca.* 0.1 g), methanol (40 mL) and carbon tetrachloride (400 mL) was heated at reflux for 18 h on an apparatus equipped with a Soxhlet extractor containing dry 4 Å molecular sieves in the thimble. The cooled solution was washed with 10% sodium carbonate solution (100 mL) and the dried (Na_2SO_4) organic phase was evaporated (in vacuo) to afford a yellow oil. The methyl ester was obtained in its pure form upon distillation, bp 125-126°/0.26 mm, (45 g, 89%) with ^{1}H-NMR δ: 1.27 (br.s, 10H), 1.37-1.45 (m, 2H), 1.57-1.65 (m, 2H), 1.83 (quint, 2H, J=7 Hz), 2.29 (t, 2H, J=7 Hz), 3.39 (t, 2H, J=7 Hz), 3.65 (s, 3H) ppm. It was used directly in the next step. A mixture of pentane-2,4-dione (10.7 g, 0.107 mol), methyl 11-bromo-undecanoate (29.8 g, 0.107 mol), anhydrous potassium carbonate (17.8 g, 0.129 mol), anhydrous cesium carbonate (1.8 g, 10 wt% of K_2CO_3) in dry acetonitrile (200 mL) and dry dimethyl sulfoxide (50 mL) was heated at 65°, under nitrogen, for 24 h. The hot reaction mixture was added to ice-cold water (300 mL) and the aqueous phase extracted with dichloromethane (3 x 100 mL). The combined organic extracts were washed with water (3 x 100 mL), dried (Na_2SO_4) and evaporated (in vacuo) to give an oily residue (30.4 g, 96%). The crude oil was dissolved in glacial acetic acid (100 mL) and heated at reflux in the presence of anhydrous sodium acetate (22 g, 0.26 mol). After 12 h, the hot solution was poured into ice-cold water (150 mL) and extracted with dichloromethane (2 x 100 mL). The combined organic extracts were washed with 10% sodium carbonate (3 x 100 mL) and dried (Na_2SO_4). Evaporation

of the dichloromethane gave an oil (30 g), which was distilled (154-156°C, 0.04 mm) to afford the title compound as a white waxy solid (20 g, 63%). It had ^{1}H-NMR, δ: 1.23 (br.s, 14H), 1.52-1.65 (m, 2H), 1.75-1.85 (m, 2H), 2.15 (s, 6H), 2.27 (t, 2H, J=7 Hz), 3.58 (t, 1H, J=7 Hz), 3.64 (s, 3H) ppm and ^{13}C-NMR, δ: 24.78 (t), 27.40 (t), 28.10 (t), 28.89 (t), 28.95 (t), 29.05 (t), 29.10 (t), 29.19 (t), 29.25 (t), 29.25 (q), 33.92 (t), 51.24 (q), 68.84 (d), 174.08 (s), 204.32 (s) ppm.

Anal. Calcd for $C_{17}H_{30}O_4$ (298.4): C, 68.42; H, 10.13.
Found: C, 68.16; H, 10.40.

2,4-Dimethyl-5(ethoxycarbonyl)-1*H*-pyrrole-3-undecanoic acid methyl ester (7): To a cold (−5°) solution of ethyl acetoacetate (9.4 g, 0.072 mol) in glacial acetic acid (100 mL) was slowly added a solution of sodium nitrite (6.9 g, 0.1 mol) in water (10 mL) at such a rate so the reaction temperature remained below 7°C (approx. 3 min.). The resultant solution was stirred at room temperature for 3 h before the addition of a solution of methyl 12-acetyl-13-oxotetradecanoate (5.4 g, 0.018 mol) in tetrahydrofuran (10 mL). This was followed by the addition of zinc dust (19.5 g, 0.3 g-atom) at such a rate so to keep the reaction temperature between 70-80°. After addition of zinc was complete, the resultant solution was heated at reflux for 14 h. The hot solution was poured into ice-cold water (250 mL) and the resultant oily aqueous phase was extracted with dichloromethane (3 x 80 mL). The combined organic phase was washed with 10% sodium carbonate (3 x 60 mL), water (100 mL) and dried (Na_2SO_4) before evaporating (in vacuo) to dryness. The title compound was obtained from the crude residue by dissolving the residue in 95% ethanol and keeping at -30°C. Filtration and reducing the mother liquor and storing at -30° afforded more title compound as an off-white solid. This procedure was repeated several times to give pure **7** in 64% yield (4.2 g). A small sample was recrystallized, from 95% ethanol, for microanalysis. It had mp 68-69°; ^{1}H-NMR δ: 1.27 (br.s, 12H), 1.35 (t, 2H, J=7 Hz), 1.39-1.48 (m, 2H), 1.59-1.65 (m, 2H), 2.19 (s, 3H), 2.26 (s, 3H), 2.3 (t, 2H, J=7 Hz), 2.34 (t, 2H, J=7 Hz), 3.67 (s, 3H), 4.29 (q, 2H, J=7 Hz), 8.47 (br.s, 1H) ppm; ^{13}C-NMR δ: 11.21 (q), 12.11 (q), 15.21 (q), 24.62 (t), 25.55 (t), 29.74 (t), 29.84 (t), 30.03 (2xt), 30.13 (t), 30.16 (t), 31.46 (t), 34.71 (t), 52.01 (q), 60.12 (t), 117.3 (s), 123.06 (s), 127.7 (s), 130.0 (s), 162.4 (s), 174.9 (s) ppm.

Anal. Calcd. for $C_{21}H_{35}N_4O$ (365.5): C, 69.00; H, 9.65; N, 3.83.
Found: C, 69.10; H, 9.89; N, 3.78.

5-[1,5-Didehydro-3-ethyl-4-methyl-5-oxo-2*H*-pyrrole-2-ylidene methyl]-2,4-dimethyl-1*H*-pyrrole-3-undecanoic Acid Methyl Ester (4): To a two-phase system of 2,4-dimethyl-5-(ethoxycarbonyl)-1*H*-pyrrole-3-undecanoic acid methyl ester (3.85 g, 0.011 mol) in ethanol (30 mL) and 50 wt% aqueous sodium nitrate (35 mL) was added solid sodium hydroxide, and the mixture was brought to reflux. After 15 min., the ethanol was allowed to evaporate, almost completely (if the reaction mixture starts to froth, then add a minimum amount of ethanol to stop frothing). The suspension was allowed to reflux for 18 h before cooling to about -30° and carefully acidified (dropwise) with a cold (about -30°) solution containing concentrated nitric acid, 50 wt% aqueous sodium nitrate and water (2:3.5:1) (65 mL). The acidified solution was filtered on a Büchner funnel, which had been stored in the freezer at -30°C., and the precipitate was washed with ice-cold water (10 mL). The brown solid (**6**) was immediately used, without further purification, in the next step. Crude 2,4-dimethyl-5-carboxy-1H-pyrrole-3-undecanoic acid (from above) was added to a solution of 5-bromomethylene 4-ethyl-3-methyl-2-oxo-1H-pyrrole (**5**) (2.75 g, 12.6 mmol) in dry methanol

(75 mL) and heated at reflux for 18 h. The resultant dark brown solution was stored at -30°C. The suspension was filtered and the solid recrystallized from methanol/chloroform to yield the dipyrrinone as a pale green powder (1.6 g, 36%). Further purification was achieved by radial chromatography (chromatotron) on silica using chloroform/methanol (95:5) as eluant. Fractions 3-5 were combined and recrystallized from methanol to afford **4** as a fluffy yellow solid, mp 107-108°. It had ϵ_{406}^{max} 33,000 (CH_2Cl_2), ϵ_{415}^{max} 34,800 (CH_3OH), ϵ_{413}^{max} 22,500 ($(CH_3)_2SO$); ^{1}H-NMR δ: 1.17 (t, 3H, J=7 Hz), 1.28 (br.s, 12H), 1.39-1.45 (m, 2H), 1.55-1.62 (m, 2H), 1.95 (s, 3H), 2.13 (s, 3H), 2.3 (t, 2H, J=7 Hz), 2.37 (br.t, 2H, J=7 Hz), 2.4 (s, 3H), 2.57 (q, 2H, J=7 Hz), 3.65 (s, 3H), 6.15 (s, 1H), 10.32 (br.s, 1H), 11.30 (br.s, 1H) ppm; ^{13}C-NMR δ: 8.50 (q), 9.68 (q), 11.69 (q), 15.07 (q), 17.97 (t), 24.28 (t), 24.97 (t), 29.16 (t), 29.27 (t), 29.39 (t), 29.48 (t), 29.59 (t), 29.61 (t), 30.98 (t), 34.12 (t), 51.43 (q), 101.3 (d), 121.5 (s), 122.1 (2xs), 124.9 (s), 126.8 (s), 131.5 (s), 148.2 (s) 174.03 (s), 174.09 (s) ppm.

Anal. Calcd. for $C_{26}H_{40}N_2O_3$ (428.6): C, 72.86; H, 9.41; N, 6.54.
Found: C, 73.13; H, 9.30; N, 6.27.

3,17-Diethyl-2,7,13,18-tetramethyl-(21*H*,24*H*)-bilin-1,19-dione-8,12-bis-undecanoic acid Dimethyl Ester [(n=10) Mesobiliverdin-XIIIα Dimethyl Ester] (3): To a refluxing solution of *p*-chloranil (1.95 g, 7.9 mmol) in dichloromethane (100 mL) was added a solution of dipyrrinone **4** (1.35 g, 3.15 mmol) in dichloromethane (25 mL). After 5 minutes at reflux, 98% formic acid (15 mL) was added, and refluxing was continued for 24 h. The reaction mixture was stored at about -30° for 14 h, and the suspension was filtered. The filtrate was washed with 2 *M* sodium hydroxide until the aqueous washings remained basic. The dichloromethane phase was collected, dried ($MgSO_4$) and evaporated to afford a black solid. Recrystallization from methanol afforded verdin **3** as dark blue needles (47%, 0.63 g), mp 124-125°. It had UV-visible, ϵ_{642}^{max} 14,400, ϵ_{369}^{max} 48,800 (benzene), ϵ_{716}^{sh} 15,200, ϵ_{659}^{max} 23,600, ϵ_{368}^{max} 52,500 ($CHCl_3$), ϵ_{641}^{max} 15,700, ϵ_{365}^{max} 55,200 (CH_3OH), ϵ_{637}^{max} 16,900, ϵ_{370}^{max} 52,100 ($(CH_3)_2SO$); ^{1}H-NMR, δ: 1.20 (t, 6H, J=7 Hz), 1.28 (br.s, 28 H), 1.48-1.55 (m, 4H), 1.55-1.65 (m, 4H), 1.81 (s, 6H), 2.05 (s, 6H), 2.92 (t, 4H, J=7 Hz), 2.48 (q, 2H, J=7 Hz), 2.54 (q, 2H, J=7 Hz), 3.49 (s, 6H), 5.91 (s, 2H), 6.61 (s, 1H) ppm; ^{13}C-NMR, δ: 8.94 (q), 10.17 (q), 15.06 (q), 18.43 (t), 25.16 (t), 25.56 (t), 29.69 (t), 29.76 (t), 29.88 (t), 30.05 (t), 30.08 (t), 30.15 (t), 32.06 (t), 34.70 (t), 52.02 (q), 97.08 (d), 114.7 (d), 128.1 (s), 128.8 (s), 140.2 (s), 140.6 (s), 142.0 (s), 147.3 (s), 150.4 (s), 173.2 (s), 174.9 (s) ppm.

Anal. Calcd. for $C_{51}H_{74}N_4O_6$ (839.2): C, 73.00; H, 8.89; N, 6.68.
Found: C, 73.15; H, 8.77; N, 7.08.

3,17-Diethyl-2,7,13,18-tetramethyl-(10*H*,22*H*,23*H*,24*H*)-bilin-1,19-dione-8,12-bis-undeconoic acid Methyl Ester [(n=10) Mesobilirubin-XIIIα Dimethyl Ester] (2): To a solution of **3** (441 mg, 0.526 mmol) in nitrogen-purged dry 2-propanol (100 mL) and nitrogen-purged dry methanol (10 mL) was added sodium borohydride (1.1 g, 29 mmol) and the resultant blue solution began to turn green then yellow as it was stirred for 1.5 h at room temperature under nitrogen. The suspension was acidified with 10% HCl solution, stored at about -30° for 2 h, then vacuum filtered. The yellow solid was dissolved in dichloromethane (150 mL) and washed with water (100 mL). The dried organic phase was evaporated to give a brown/yellow solid. Flash chromatography (TLC grade silica +15 wt% water, dichloromethane as eluant) yielded a yellow solid which was recrystallized from chloroform/methanol to give **2** as a yellow powder (238 mg, 54%) mp 192-3°C (dec). It had UV-visible in Table 2; ^{1}H-NMR, δ: 1.0 (t, 6H, J=7 Hz), 1.29

(br.s, 24H), 1.43-1.48 (m, 4H), 1.47 (br.s, 6H), 1.6-1.64 (m, 4H), 2.08 (s, 6H), 2.28-2.34 (m, 8H), 2.47-2.51 (m, 4H), 3.66 (s, 6H), 4.09 (s, 2H), 5.93 (s, 2H), 10.31 (s, 2H), 10.63 (s, 2H) ppm; ^{13}C-NMR, δ: 8.46 (q), 10.19 (q), 15.31 (q), 18.21 (t), 25.11 (t), 25.43 (t), 29.61 (t), 29.77 (t), 29.95 (t), 30.12 (t), 30.14 (t), 30.28 (t), 31.78 (t), 34.54 (t), 51.84 (q), 100.5 (d), 121.7 (s), 123.5 (s), 123.8 (s), 123.8 (s), 128.7 (s), 131.5 (s), 147.7 (s), 174.34 (s), 174.65 (s) ppm.

Anal. Calcd for $C_{51}H_{76}N_4O_6$ (841.2): C, 72.82; H, 9.10; N, 6.66.
Found: C, 72.66; H, 9.03; N, 6.85.

3,17-Diethyl-2,7,13,18-tetramethyl-(10*H*,22*H*,23*H*,24*H*)-bilin-1,19-dione-8,12-bis-undecanoic acid [(n=10) Mesobilirubin-XIIIα] (1): To a refluxing solution of **2** (0.44 g, 0.524 mmol) in nitrogen-purged, dry tetrahydrofuran (50 mL) and nitrogen-purged, dry methanol (8 mL) was added nitrogen-purged 0.75 *M* NaOH (8 mL), and refluxing was continued for 3 h under nitrogen. The yellow suspension was acidified with 10% HCl, stored at about -30° for 1 h then vacuum filtered. The pale green solid was kept under vacuum (oil pump) for approx. 12 h to remove water. The dry solid was dissolved in dichloromethane and chromatographed (chromatotron, silica, dichloromethane/methanol 94:6 as eluant). The yellow solid was collected and recrystallized from dichloromethane/methanol. Final purification was achieved by centrifugal chromatography using dichloromethane/methanol 94:6 as the eluant. Pure **1** had mp 190° (dec); UV-visible in Table 2; ^{1}H-NMR (d_6-DMSO) δ: 0.97 (t, 6H, J=7 Hz), 1.13 (br.s, 28H), 1.54 (br.s, 6H), 1.98 (s, 6H), 2.15 (t, 4H, J=7 Hz), 2.30 (br.m, 12H), 3.96 (s, 2H), 5.86 (s, 2H), 10.01 (s, 2H), 10.27 (s, 2H), 11.46 (br.s, 2H) ppm; ^{13}C-NMR 6.53 (q), 7.75 (q), 13.27 (q), 15.97 (t), 22.35 (t), 22.73 (t), 23.15 (t), 27.30 (t), 27.55 (t), 27.67 (t), 27.87 (tx2), 28.17 (t), 29.04 (t), 32.36 (t), 96.59 (d), 119.8 (s), 120.37 (s), 121.07 (s), 121.56 (s), 125.8 (s), 128.9 (s), 145.6 (s), 170.9 (s), 173.3 (s) ppm.

Anal. Calcd for $C_{49}H_{72}N_4O_6$ (813.1): C, 72.38; H, 8.92; N, 6.89.
Found: C, 72.14; H, 9.06; N, 7.26.

Molecular mechanics calculations and molecular modelling was carried out on an Evans and Sutherland ESV-10 workstation using version 5.41 of SYBYL (Tripos Assoc., St. Louis, MO). The dipyrrinone units of **1** were rotated independently about the central -CH_2- at C_{10} (Torsion angles ϕ_1 and ϕ_2) through 10° increments from 0° to 360°. (The $\phi_1 = 0°$, $\phi_2 = 0°$ conformer has a porphyrin shape.) In this procedure, the two torsion angles were held fixed at each increment while the remainder 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 set of ϕ values.

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