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Synthesis and Properties of a Lipid Bilirubin Analog

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Abstract: A new synthesis of dipyrrinone acids is described from the known dipyrrinone, 3-ethyl-2,7,9-trimethyl-(10*H*)-dipyrrin-1-one (**11**) with a free β-position. In six steps, **11** was converted to **5** with a C_{20} eicosanoic acid chain, and **5** was converted to a lipid analog (**1**) of bilirubin with eicosanoic acids replacing propionic at carbons C(8) and C(12). Unlike bilirubins with short acid chains, **1** behaves much like its dimethyl ester (**2**) and probably adopts a helical rather than the characteristic ridge-tile conformation. Circular dichroism of **1** complexed with quinine in $CHCl_3$ gives a bilirubin-like bisignate Cotton effect: $\Delta\epsilon_{438}^{max}$ +18, $\Delta\epsilon_{390}^{max}$ −24.

INTRODUCTION

Linear tetrapyrroles such as the bilirubin and biliverdin (Fig. 1) are formed in animal metabolism from normal turnover of hemoglobin and other heme proteins.[1,2,3] Pigment conformation seems to dictate many of their properties. Biliverdin adopts a helical, porphyrin-like shape;[1] bilirubin does not, although its dimethyl ester probably does.[1] Recent efforts to understand the properties and metabolism of bilirubin have focussed on its unique ability to adopt a ridge-tile conformation where the carboxylic acid groups are involved in intramolecular hydrogen bonding.[4,5,6] This makes bilirubin lipophilic and not excretable in normal metabolism, except by glucuronidation.[2,3] Recently it has become evident that translocation of the propionic acid groups away from the natural locations at C(8) and C(12) leads to pigments which are more polar than bilirubin and do not require glucuronidation for excretion.[7] However, analogs with propionic acid groups at C(8) and C(12), *e.g.*, mesobilirubin-XIIIα, typically exhibit the same unique polarity and inexcretability

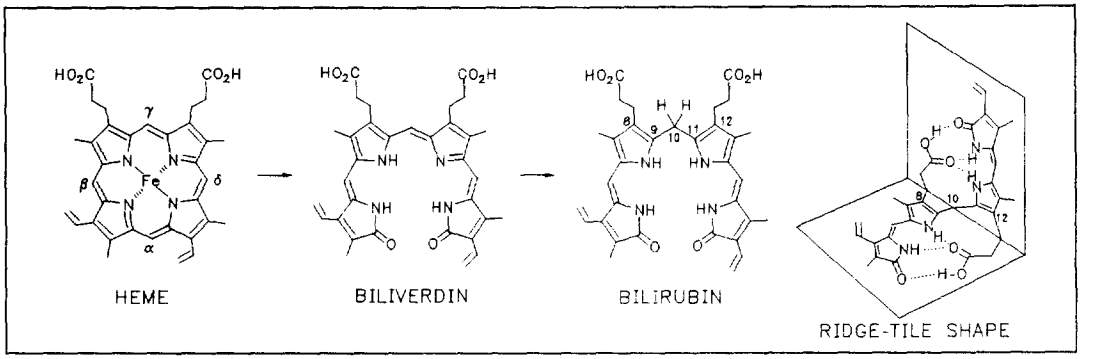
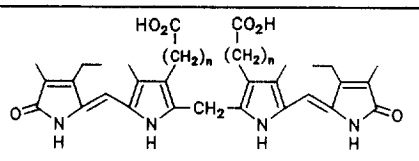


FIGURE 1. Conversion of heme to bilirubin shown (left) in a porphyrin-like conformation and (right) in the energetically most stable, intramolecularly hydrogen-bonded ridge-tile conformation.

properties as bilirubin. For these pigments can also tuck their carboxylic acid groups inward, where they are tethered to an opposing dipyrrinone by intramolecular hydrogen bonding.

Such intramolecular hydrogen bonding is one of the most interesting and important facets of bilirubin structure.[1,4-8] Although the two component dipyrrinone units of bilirubin-type molecules may rotate relatively freely and independently about the interconnecting C(10) -CH_2- group, the ridge-tile conformation shown is stabilized through a unique and extensive network of intramolecular hydrogen bonds. In earlier studies,[9,10,11] we investigated the potential for intramolecular hydrogen bonding in bilirubin analogs with shorter and longer alkanoic acid chains, relative to propionic acid, at C(8) and C(12). Evidence for intramolecular hydrogen bonding was found for acetic and through hexanoic acid chains,[10] and even in rubins with undecanoic acid chains.[11] As the alkanoic acid chains lengthen beyond hexanoic, the pigment core of the molecule was able to adopt new folded conformations while retaining hydrogen bonding.[11] The net result was that helical, ridge-tile and stretched conformations[6] became comparable in energy.[11] Our interest in pigment stereochemistry stabilized by intramolecular hydrogen bonding between alkanoic acid and dipyrrinone groups led us to consider: (i) whether such hydrogen bonding might be retained in a bilirubin analog where both propionic acid groups are replaced by very long alkanoic acid groups, and (ii) 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 lipid-like symmetric bilirubin analog (**1**), with eicosanoic acid groups replacing the conventional propionic acids of mesobilirubin-XIIIα.

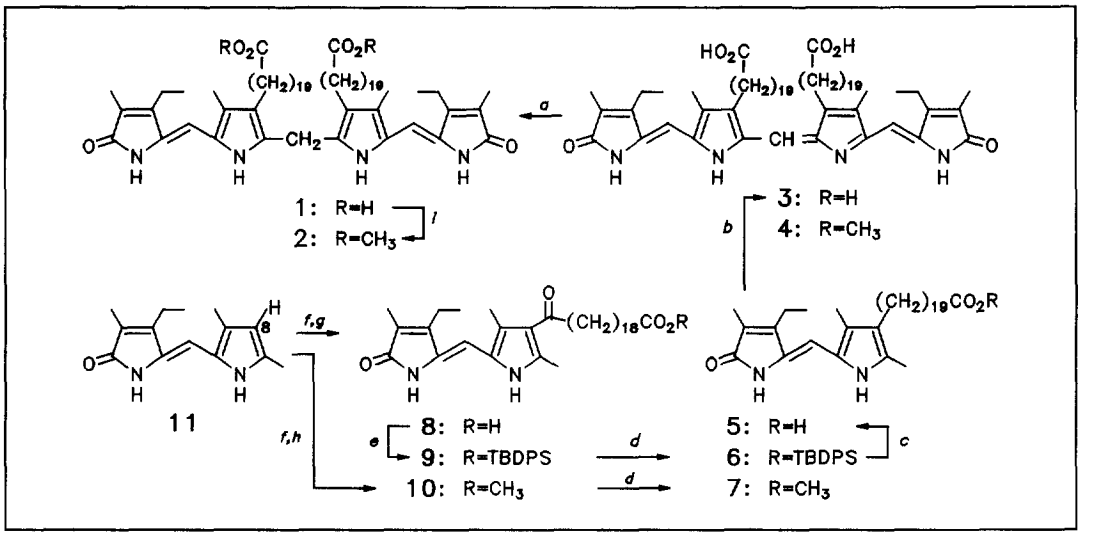


1: (n=19) Mesobilirubin-XIIIα (with eicosanoic acids)
(n=2) Mesobilirubin-XIIIα (with propionic acids)
(n=10) Mesobilirubin-XIIIα (with undecanoic acids)

RESULTS AND DISCUSSION

Synthesis. The target bilirubin, (n=19) mesobilirubin-XIIIα (**1**), was reached through the series of steps outlined in the Synthetic Scheme. Thus, the easily prepared known dipyrrinone **11**[12] was reacted in CH_2Cl_2-CH_3NO_2 at the vacant C(8) site with the diacid chloride of eicosanoic acid under mild Friedel-Crafts acylation[13] conditions using anhydrous $SnCl_4$ catalyst. With an aqueous work-up, the dipyrrinone keto-acid (**8**) was obtained; work-up with methanol gave the keto-methyl ester (**10**), and the isolated yields were 76-78% in either case. The keto group of **10** was reduced selectively with borane-tetrahydrofuran[13] to give dipyrrinone ester (**7**) in 89% yield. Oxidative coupling of **7** gave the (n=19) mesobiliverdin-XIIIα (**4**), from which the (n=19) mesobilirubin-XIIIα (**2**) could be obtained following reduction with $NaBH_4$. Attempts to saponify either **2** or **4** were only partially successful, and the mixture of acids and esters proved inseparable. Similar difficulties were encountered in attempts to saponify the dipyrrinone ester **7**. This led us to consider the more easily removed phenyldimethylsilyl group as an alternative to the methyl ester for protecting the acid of **8** during the borane-tetrahydrofuran reduction of its keto group. Thus, **8** was converted to **9**, which was reduced to **6** in 92% yield. Deprotection of **6** gave **5**, which could be oxidatively coupled to give the (n=19) mesobiliverdin-XIIIα (**3**) as a dark blue solid in 45% isolated yield. Reduction of **3** with $NaBH_4$ afforded the desired (n=19) mesobiliverdin-XIIIα (**1**) as a waxy off-yellow solid in 59% yield.

SYNTHETIC SCHEME



[a] $NaBH_4/(CH_3)_2CHOH$ (59%); [b] *p*-chloranil/CH_2Cl_2-HCO_2H, refl. (45%); [c] *n*-$Bu_4N^+F^-$/THF (60%); [d] B_2H_6/THF 0°C (92%); [e] *tert*-butyldiphenylsilylchloride (TBDPSCl), imidazole/THF (52%); [f] eicosanedioic acid dichloride, $SnCl_4/CH_2Cl_2$-CH_3NO_2; [g] H_2O (78%); [h] CH_3OH (76%); [i] CH_2N_2.

Polarity from Chromatographic Behavior. The (n=19) mesobilirubin-XIIIα (**1**) has a much longer retention time (>2 hrs.) when coinjected with the (n=10) mesobilirubin-XIIIα analog (~19.7 minutes) or with the parent (n=2) mesobilirubin-XIIIα (~11.4 minutes) on reverse phase HPLC. These results suggest that **1** is much more lipophilic than either (n=2) or (n=10) mesobilirubin-XIIIα. However, on silica gel TLC, **1** has a larger R_f value (0.66) when compared to (n=10) mesobilirubin-XIIIα (R_f=0.0) and a much smaller R_f value compared with the (n=2) mesobilirubin-XIIIα standard (R_f=0.87) using CH_2Cl_2:CH_3OH (9:1, vol/vol) as eluant.

^{13}C-NMR and Structure. The tetrapyrrole core structure of **1** is the same as that of its (n=2) and (n=10) mesobilirubin-XIIIα analogs and therefore exhibits essentially the same ^{13}C-NMR chemical shifts (Table 1). In addition, **1** exhibits a long string of high field signals due to the ethyl, methyl and $(CH_2)_{19}$ substituents.

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.[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 mesobilirubin-XIIIα) when the dipyrrinone and carboxylic acid groups are intramolecularly hydrogen bonded[14,15] When (n=2) mesobilirubin-XIIIα 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 2).[16,17] 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.

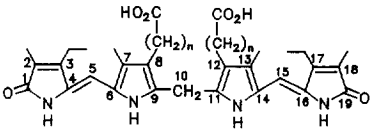


TABLE 1. Comparison of ^{13}C-NMR Chemical Shifts of Carboxyl Carbons and Tetrapyrrole Core of (n=19) Mesobilirubin-XIIIα (**1**) and (n=10) Mesobilirubin-XIIIα With the Parent, (n=2) Mesobilirubin-XIIIα.

Carbon	δ (ppm) in $(CD_3)_2SO$		
	n=19 (**1**)	n=10	n=2
1, 19	171.8 (s)	170.9 (s)	171.9 (s)
2, 18	122.7 (s)	121.6 (s)	122.9 (s)
3, 17	147.0 (s)	145.1 (s)	147.1 (s)
4, 16	127.1 (s)	125.8 (s)	127.8 (s)
5, 15	97.7 (d)	96.6 (d)	97.7 (d)
6, 14	122.5 (s)	121.0 (s)	122.4 (s)
7, 13	121.5 (s)	120.4 (s)	122.0 (s)
8, 12	121.0 (s)	119.8 (s)	119.2 (s)
9, 11	130.4 (s)	128.9 (s)	130.9 (s)
10	24.14 (t)	23.15 (t)	23.34 (t)
CO_2H	174.4 (s)	173.3 (s)	174.1 (s)

TABLE 2. Comparison of Rubin Lactam and Pyrrole N-*H* Chemical Shifts[a] in $CDCl_3$ and $(CD_3)_2SO$.[b]

Pigment	δ (ppm) in $CDCl_3$			δ (ppm) in $(CD_3)_2SO$		
	Lactam	Pyrrole	CO_2H	Lactam	Pyrrole	CO_2H
(n=19) Mesobilirubin-XIIIα (**1**)	10.57	10.10	11.51	9.80	10.33	11.90
(n=10) Mesobilirubin-XIIIα	10.53	9.20	13.56	9.79	10.23	11.88
(n=2) Mesobilirubin-XIIIα	10.57	9.15	13.62	9.72	10.27	11.91
(n=19) Mesobilirubin-XIIIα Dimethyl Ester (**2**)	10.63	10.30	—	Insol.	Insol.	—
(n=10) Mesobilirubin-XIIIα Dimethyl Ester	10.63	10.30	—	9.72	10.38	—
(n=2) Mesobilirubin-XIIIα Dimethyl ester	10.54	10.27	—	9.74	10.40	—
Etiobilirubin-IVγ	10.58	10.28	—	9.78	10.28	—

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

As was anticipated, the pyrrole (and lactam) N-*H* chemical shifts of **1**, (n=2) and (n=10) mesobilirubin-XIIIα, are the same in $(CD_3)_2SO$ solvent (Table 2). In the latter two rubins, the data in $CDCl_3$ reveal a pyrrole N-*H* chemical shift near 9.2 δ characteristic of an intramolecularly hydrogen bonded ridge-tile conformation where a pyrrole N-H lies over a neighboring pyrrole ring and is shielded by ring current anisotropy.[18] In contrast, the pyrrole NH of **1** is much more deshielded, suggesting a conformation where the pyrrole N-H's do not lie above the opposing pyrrole rings. Whether the CO_2H groups of **1** are still tethered to the dipyrrinones is not clear from the data. Molecular mechanics calculations indicate a stable intramolecularly hydrogen bonded *helical* structure, but a helical conformation without hydrogen bonding is also possible. Both **1** and

its dimethyl ester (**2**) exhibit nearly the same N-*H* chemical shifts in $CDCl_3$. Similar sets of chemical shifts have been observed in bilirubin and mesobilirubin dimethyl ester and etiobilirubin[14,15,17] and have been correlated with intermolecular dipyrrinone-dipyrrinone hydrogen bonding for rubins in a helical conformation.

UV-Visible Spectral Analysis and Conformation from Exciton Coupling. Further evidence on conformation 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 (n=2) mesobilirubin-XIIIα change very little, with λ^{max} being near 430 nm and λ^{sh} near 395 nm[10,14,17] — corresponding to the two exciton components[19] from electric transition dipole-dipole interaction of the two proximal dipyrrinone chromophores approximately 90° apart.[6] Since (n=2) mesobilirubin-XIIIα is known from NMR studies to adopt molecularly hydrogen-bonded ridge-tile conformation in $CDCl_3$ solvent, 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. The UV-visible spectra of **1** (Table 3) are very much like those of (n=10) mesobilirubin-XIIIα and less like those of (n=2) mesobilirubin-XIIIα. For example, in $CHCl_3$, λ^{max} is now at 389 nm, with λ^{sh} at 429 nm, whereas, the reverse is the case for (n=2) mesobilirubin-XIIIα. The data for **1** are consistent with a folded rather than a stretched conformation (but not a ridge-tile conformation), probably involving hydrogen bonding in $CHCl_3$ and other non-polar solvents, with the conformation deformed to a smaller dihedral angle[6] giving the pigment a helical shape.

TABLE 3. Comparison of UV-Visible Spectroscopic Data for (n=19, 10 and 2) Mesobilirubin-XIIIα and Their Dimethyl Esters.[a]

Pigment	$CHCl_3$		THF		$(CH_3)_2SO$	
	ϵ^{max}	(λ, nm)	ϵ^{max}	(λ, nm)	ϵ^{max}	(λ, nm)
(n=19) Mesobilirubin-XIIIα (**1**)	32,100 49,000	$(429)^{sh}$ (389)	32,900 60,200	$(421)^{sh}$ (381)	58,100 40,000	(434) $(398)^{sh}$
(n=10) Mesobilirubin-XIIIα	33,500 47,900	$(430)^{sh}$ (395)	Insol	Insol	61,600 40,000	(434) $(390)^{sh}$
(n=2) Mesobilirubin-XIIIα	57,800	(431)	55,900	429	57,000 49,000	(426) $(397)^{sh}$
(n=19) Mesobilirubin-XIIIα Dimethyl Ester (**2**)	19,700 56,000	$(429)^{sh}$ (382)	32,600 54,000	$(419)^{sh}$ (383)	Insol	Insol
(n=10) Mesobilirubin-XIIIα Dimethyl Ester	19,900 72,600	$(430)^{sh}$ (380)	29,800 60,100	$(422)^{sh}$ (381)	71,500 45,800	(436) $(400)^{sh}$
(n=2) Mesobilirubin-XIIIα Dimethyl Ester	59,300	(382)	38,000 51,800	$(412)^{sh}$ (379)	61,500 42,800	(430) $(396)^{sh}$

[a] Run at 1 x 10^{-5} M concentration; ϵ in M^{-1} cm^{-1}.

The UV-visible spectral solvent dependence of dimethyl ester **2** is very similar to that of parent acid **1** (Table 3), as well as to (n=10) and (n=2) mesobilirubin-XIIIα dimethyl ester. The last typically exhibits a strong solvent, concentration and temperature dependence due to formation of dimers[1,20] in non-polar solvents such as THF and $CHCl_3$. This results in spectra with a narrow bandwidth, intense absorption at λ^{max}

near 380 nm and a weak shoulder at λ^{sh} near 430 nm.[1,10] In more polar solvents such as $(CH_3)_2SO$, the solutions are largely monomeric, and the UV-visible spectra are somewhat similar to those of the parent acid in these solvents, with λ^{max} near 435 nm and λ^{sh} near 400 nm.[10,15a] The intermolecularly hydrogen bonded pigments probably adopt folded helical structures with smaller dipyrrinone-dipyrrinone (interplanar) dihedral angles.[5a,6,14] The UV-visible spectra of **2** are thus entirely consistent with the expectations drawn from earlier studies of mesobilirubin and bilirubin dimethyl esters.[14] They are also quite similar to those of **1**. Intermolecular hydrogen bonding would appear to predominate in both **1** and **2**.

UV-Visible Spectra of Verdins. The UV-visible spectral data of **3** and **4** and corresponding (n=10) and (n=2) verdin acids and esters are given in Table 4. No significant differences can be found between acids and esters or between esters and acids of differing alkanoic acid chain lengths. The data are consistent with helical, porphyrin-like conformations similar to that favored in biliverdin and its analogs.[1]

TABLE 4. UV-Visible Spectral Data for (n=19, 10 and 2) Mesobiliverdins and Their Methyl Esters.[a]

Pigment	$CHCl_3$		THF		$(CH_3)_2SO$	
	ϵ^{max}	(λ, nm)	ϵ^{max}	(λ, nm)	ϵ^{max}	(λ, nm)
(n=19) Mesobiliverdin-XIIIα (**3**)	12,400 45,200	639 371	15,500 48,600	634 366	15,000 42,300	654 371
(n=10) Mesobiliverdin-XIIIα	12,800 42,000	642 369	16,700 44,700	635 367	12,800 42,000	642 369
(n=2) Mesobiliverdin-XIIIα	10,700 32,200	634 366	14,400 45,600	627 364	13,400 44,700	635 368
(n=19) Mesobiliverdin-XIIIα Dimethyl Ester (**4**)	11,900 44,600	638 369	16,000 45,600	635 366	Insol	Insol
(n=10) Mesobiliverdin-XIIIα Dimethyl Ester	10,800 44,800	631 369	15,100 49,300	638 367	17,900 45,600	662 370
(n=2) Mesobiliverdin-XIIIα Dimethyl Ester	13,000 46,100	634 369	16,100 45,600	630 364	21,400 46,000	664 369

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

Dipyrrinone Dimers. Dipyrrinones, including esters are known to form tight, intermolecularly hydrogen bonded dimers in non-polar solvents such as $CHCl_3$,[11] but in dimethylsulfoxide dipyrrinones are thought to be monomeric and hydrogen bonded to solvent. Characteristic ^{1}H-NMR chemical-shifts of the pyrrole and lactam hydrogens distinguish the dimer from the monomer $(CD_3)_2SO$ complex. Not surprisingly, in $(CD_3)_2SO$, the N*H* chemical shifts of acid **1** are nearly the same as its ester, as are those of (n=10) and (n=2) mesobilirubin-XIIIα. However, in $CDCl_3$, the acids and esters differ markedly, with the pyrrole N-H experiencing a strong (1-1.5 ppm) shielding.

Induced Circular Dichroism. As may be seen in Fig. 2, a solution of **1** in $CHCl_3$ solution containing a 300:1 molar ratio of quinine:pigment gives a well-defined, strong bisignate circular dichroism (CD) spectrum for the long wave-length UV-visible transition. The CD is opposite in sign but similar in magnitude to that

TABLE 5. Comparison of Lactam and Pyrrole N-*H* and Carboxylic Acid O*H* ^{1}H-NMR Chemical Shifts[a] of Dipyrrinones in $CDCl_3$[b] and $(CD_3)_2SO$[c] Solvents.

Dipyrrinone	$CDCl_3$			$(CD_3)_2SO$		
	Lactam	Pyrrole	CO_2H	Lactam	Pyrrole	CO_2H
(n=19) Acid (**8**)	10.70	9.01	13.39	9.71	10.21	11.91
(n=10) Acid	10.45	8.64	12.43	9.39	10.37	11.95
(n=2) Acid	9.90	8.84	13.60	9.75	10.27	12.03
(n=19) Methyl Ester (**10**)	10.70	9.94	—	9.71	10.21	—
(n=10) Methyl Ester	10.86	10.05	—	9.75	10.24	—
(n=2) Methyl Ester	10.92	10.13	—	9.72	10.26	—

[a] δ, ppm downfield from $(CH_3)_4Si$. [b] 10^{-3} M solutions. [c] 10^{-2} M solutions.

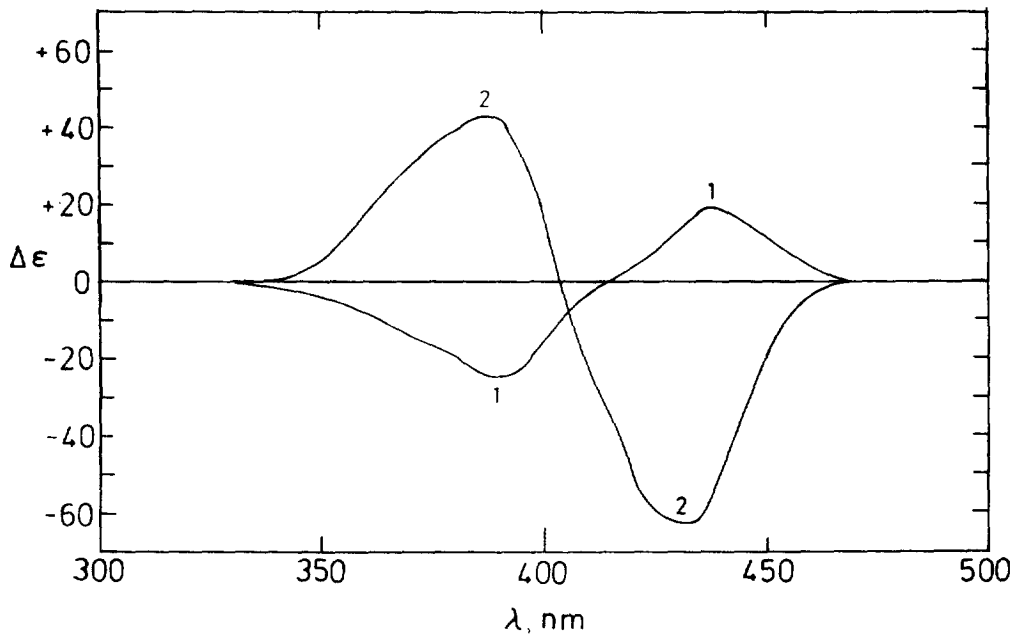


FIGURE 2. Comparison of the circular dichroism (CD) spectra of (n=19) mesobilirubin-XIIIα (**1**) (curve 1) (n=2) mesobilirubin-XIIIα (curve 2) in $CHCl_3$ with quinine (300:1 molar ratio of quinine:pigment) at 22°C. The concentration of pigment in each spectrum is 2 x 10^{-4} *M* and that of quinine is 0.06 *M*.

observed for (n=2) mesobilirubin-XIIIα.[21] Bilirubin and (n=2) mesobilirubin-XIIIα are known to exist as a 1:1 mixture of enantiomeric ridge-tile conformers of ***M*** and ***P*** helicity.[6] (The ***M*** helicity conformer is shown in Fig. 1.) In solutions with quinine (Q) the equilibrium $Q{\cdot}\boldsymbol{M} \rightleftarrows Q{\cdot}\boldsymbol{P}$ is no longer evenly balanced, and there is an excess of one enantiomeric pigment conformation, with a net resulting CD for the pigment's long wavelength transition. Interestingly, (n=10) mesobilirubin-XIIIα gives no CD under the same

conditions, but the reasons for this difference are unclear. Human serum albumin (HSA) is also known to act as chiral complexation agent for bilirubin, (n=2) and (n=10) mesobilirubin-XIIIα. When bound to HSA and other species' serum albumin, these pigments are known to exhibit optical activity, seen typically as an induced circular dichroism (CD), which is usually intense and bisignate (Table 6).[22,23] In contrast, only very weak induced CDs are found with analogs having no acid groups, *e.g.*, etiobilirubin-IVγ. The protein apparently acts as an enantioselective binding agent for bilirubins with carboxylic acid groups and constrains the pigment to adopt a chiral conformation. And it is in such a chiral conformation, whether selected by HSA or quinine, that the induced CD occurs through intramolecular exciton coupling between the dipyrrinone chromophores of the rubins. However, unlike bilirubin, (n=2) mesobilirubin-XIIIα, or even (n=10) mesobilirubin-XIIIα, no CD is found for (n=19) mesobilirubin-XIIIα in the presence of HSA. The long acid chain and lipid character of pigment **1** apparently have little influence on the enantioselection. Alternatively, pigment **1** may adopt a unique chiral conformation where its component dipyrrinone electric dipole transition moments are parallel, a situation in which exciton coupling CD is zero.[6]

TABLE 6. Comparison of Circular Dichroism and UV-visible Spectral Data[a] for (n=19) (**1**), (n=10) Mesobilirubin-XIIIα, Bilirubin and Etiobilirubin-IVγ in *p*H 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=19) Mesobilirubin-XIIIα (**1**)	<0.1	—	<0.1	54,800 (441)
(n=10) Mesobilirubin-XIIIα	+33 (449)[c]	418	-47 (396)[c]	41,900 (450) 29,000[sh] (389)
(n=2) Mesobilirubin-XIIIα	+49 (449)[d]	407	−41 (390)[d]	49,600 (436)
Bilirubin	+50 (449)	425	-24 (400)	44,000 (452)
Etiobilirubin-IVγ	+2.1 (438)	414	-3.5 (388)	22,600[sh] (432) 37,300 (375)

[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]In 3% DMSO.

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.[6] The current study shows that even when the propionic acid chains are expanded to eicosanoic acid (C_{20}), intramolecular hydrogen bonding persists in non-polar solvents. Such rubins are much more lipid-like than the parent bilirubin and are not constrained to adopt a ridge-tile shape. The C_{20} alkanoic acid chains of **1** can accommodate intramolecular hydrogen bonding in new conformations, where the dipyrrinones are rotated out of the typical ridge-tile shape and into a helical porphyrin-like conformations.

EXPERIMENTAL

General Procedures. All Ultraviolet-visible spectra were recorded on a Perkin-Elmer 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 layers). Flash column chromatography was carried out using Woelm silica gel F, thin layer chromatography grade. 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). Eicosanedioic acid was from TCI America, Inc. Stannic chloride, tert-butyl diphenylsilyl chloride, tetrachloro-1,4-benzoquinone (*p*-chloranil), quinine, tetrahydrofuran, 98% formic acid, dimethylsulfoxide and sodium borohydride were from Aldrich. Tetrahydrofuran was dried by distillation from sodium. Solutions of (n=19) mesobilirubin-XIIIα (**1**) and its dimethyl ester (**2**), (n=10) mesobilirubin-XIIIα and its dimethyl ester bilirubin-IXα and etiobilirubin-IVγ in pH 7.4 aqueous human serum albumin were prepared as reported earlier,[23] 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.

3-Ethyl-2,7,9-trimethyl-(10*H*)-dipyrrin-1-one-8-(19-oxo)eicosanoic acid (8). Eicosanedioic acid (1.50 g, 4.38 mmol) was heated at reflux in thionyl chloride (20 mL) for 30 minutes. Excess thionyl chloride was removed at water aspirator pressure (rotovap), and the crude diacid chloride was triturated twice with CCl_4. The residue was added to a suspension of 500 mg (2.17 mmol) of dipyrrinone **11** in 50 mL of CH_2Cl_2 mixed with 50 mL of CH_3NO_2 (500 mL 3-neck round-bottom flask, nitrogen inlet). Anhydrous stannic chloride (4.00 mg, 15.3 mmol) was added in two portions (second portion after two hours), and the mixture was stirred for 6.5 hours at room temperature under a steady stream of N_2. Then 200 mL of H_2O were added, and the resulting emulsion was heated at reflux for 2 hours. After cooling to room temperature, the mixture was poured into 200 mL of H_2O and extracted first with 2 x 200 mL of CH_2Cl_2 and then with 3 x (mixture of 100 mL of CH_2Cl_2 - 50 mL of CH_3OH) to break up the emulsion. The combined organic layers were dried over anhydr. Na_2SO_4, and the solvent was evaporated (rotovap). The crude product was adsorbed on silica gel and purified by flash chromatography (silica gel not deactivated: CH_2Cl_2-CH_3OH, 100:5 by vol.). The solvent was removed at aspirator pressure (rotovap), and the remaining solid was washed with hot CH_3OH. After cooling the product was filtered to give 936 mg (78% yield). It had mp 115-119° (dec.); ^{1}H-NMR, δ: 1.18 (3H, t, J=7.5 Hz), 1.26 (30H, m), 1.63-1.73 (2H, m), 1.93 (3H, s), 2.38-2.42 (5H, m), 2.55 (2H, q), 2.68-2.76 (5H, m), 5.15 (1H, s), 9.65 (1H, s), 10.83 (1H, s) ppm; ^{13}C-NMR (d_6-DMSO), δ: 8.39 (q), 12.07 (q), 14.84 (q), 15.01 (q), 17.38 (t), 24.18 (t), 24.74 (t), 28.80 (t), 28.99 (t), 29.05 (t), 29.18 (t), 29.27 (t), 33.90 (t), 42.03 (t), 96.55 (d), 121.9 (s), 122.8 (s), 122.9 (s), 124.6 (s), 130.8 (s), 137.9 (s), 147.6

(s), 172.4 (s), 179.7 (s), 196.9 (s) ppm.

Anal. Calcd for $C_{24}H_{54}N_2O_4$ (554.81): C, 73.61; H, 9.81; N, 5.05.
Found: C, 73.54; H, 9.65; N, 5.01.

3-Ethyl-2,7,9-trimethyl-(10*H*)-dipyrrin-1-one-8-eicosanoic acid (5). Dipyrrinone **6** was first protected as its *tert*-butyldiphenylsilyl ester. Thus, acyldipyrrinone **8** (217 mg, 0.391 mmol) was dissolved in 30 mL of dry tetrahydrofuran. Then 100 mg (1.47 mmol) of imidazole and 200 mg (0.727 mmol) of *tert*-butyldiphenylsilyl chloride (TBDPSCl) was added, and the mixture was stirred overnight at room temperature. Afterwards 50 mL of CH_2Cl_2 was added, and the solution was extracted with 100 mL of H_2O and dried over anhydr. Na_2SO_4. The solvent was evaporated (rotovap), and the residue was purified by flash chromatography (silica gel not deactivated: CH_2Cl_2-CH_3OH, 100:2 by vol.). Recrystallization from CH_2Cl_2-CH_3OH gave 160 mg (52%) of **9**, which was reduced directly. It had ^{1}H-NMR, δ: 1.10 (9H, s), 1.19 (3H, t, J=7.5 Hz), 1.22-1.33 (28H, m), 1.54-1.59 (2H, m), 1.64-1.71 (2H, m), 1.95 (3H, s), 2.40 (3H, s), 2.45 (2H, t, J=7.5 Hz), 2.57 (2H, q, J=7.2 Hz), 2.70-2.76 (5H, m), 6.18 (1H, s), 7.34 (6H, m), 7.34-7.43 (6H, m), 7.67 (4H, m), 10.60 (1H, s), 11.25 (1H, s) ppm; ^{13}C-NMR, δ: 9.27 (q), 11.38 (q), 14.79 (q), 15.12 (q), 17.74 (t), 18.96 (s), 24.24 (t), 24.96 (t), 26.72 (q), 26.76 (q), 28.99 (t), 29.05 (t), 29.30 (t), 29.43 (t), 29.53 (t), 35.99 (t), 42.63 (t), 100.0 (d), 122.4 (d), 122.8 (s), 123.6 (s), 125.9 (s), 127.4 (d), 128.9 (s), 129.8 (d), 131.8 (s), 135.0 (s), 139.5 (s), 148.8 (s), 174.4 (s), 197.9 (s) ppm.

The keto group of silyl ester **9** was reduced to give dipyrrinone **6**. Thus, 200 mg (0.252 mmol) of acyldipyrrinone TBDPS ester were dissolved in 40 mL of dry tetrahydrofuran and cooled to 0°C. Then 12 mL of B_2H_6 · THF complex was added in three portions, each after 1 hour reaction time. After stirring an additional hour at 0°C, the reaction mixture was diluted with 100 mL of CH_2Cl_2 and extracted with 100 mL of H_2O, then 100 mL of sat. aq. $NaHCO_3$ solution and dried over anhydr. Na_2SO_4. The solvent was evaporated to yield an oily residue, which was flash chromatographed on silica gel (not deactivated) eluting with CH_2Cl_2-CH_3OH (100:2 by vol.) to give 181 mg (92%) of ester **6**. It had ^{1}H-NMR, δ: 1.14 (9H, s), 1.20 (3H, t, J=7.8 Hz), 1.23-1.40 (30H, m), 1.50-1.60 (2H, m), 1.65-1.77 (2H, m), 1.97 (3H, s), 2.16 (3H, s), 2.38-2.43 (5H, m), 2.49 (2H, t, J=7.2 Hz), 2.55 (2H, q, J=7.8 Hz), 6.18 (1H, s), 7.41 (6H, m), 7.72 (4H, m), 10.42 (1H, s), 11.40 (1H, s) ppm; ^{13}C-NMR, δ: 8.38 (q), 9.54 (q), 11.55 (q), 14.95 (q), 17.81 (t), 19.00 (s), 24.18 (t), 25.00 (t), 26.78 (t), 29.03 (t), 29.20 (t), 29.38 (t), 29.58 (t), 30.86 (t), 36.02 (t), 101.2 (d), 121.3 (s), 121.9 (s), 122.0 (s), 124.8 (s), 126.5 (s), 127.5 (s), 129.8 (s), 131.4 (s), 131.9 (s), 135.2 (s), 148.1 (s), 172.9 (s), 173.8 (s) ppm.

Deprotection of dipyrrinone silyl ester **6** gave dipyrrinone acid **5**. Thus, 181 mg (0.232 mmol) of dipyrrinone TBDPS ester (**6**) were dissolved in 40 mL of dry THF, and 1 mL of tetra-*n*-butylammonium fluoride (1 M in THF) was added. The mixture was stirred for 1 hour at room temperature, then diluted with 100 mL of CH_2Cl_2 and extracted with 100 mL of H_2O, dried over anhydr. Na_2SO_4 and evaporated (rotovap). The resulting oily residue solidified upon adding 10 mL of CH_3OH. After cooling to -10°C the solid was filtered and dried to give 75 mg (60%). The product was used in the next step without further purification. An analytical sample was recrystallized from CH_3OH. It had mp 100-104°C (dec.); ^{1}H-NMR, δ: 1.16 (3H, t, J=7.5 Hz), 1.19-1.37 (32H, m), 1.50-1.75 (2H, m), 1.91 (3H, s), 2.11 (3H, s), 2.33-2.42 (7H, m), 2.53 (2H, q, J=7.8 Hz), 6.14 (1H, s), 9.27 (1H, s), 10.85 (1H, s) ppm; ^{13}C-NMR, δ: 8.04 (q), 9.28 (q), 11.02 (q), 14.82 (q), 17.14 (t), 23.58 (t), 24.46 (t), 28.57 (t), 28.72 (t), 28.88 (t), 28.96 (t), 28.99 (t), 30.41 (t), 33.61 (t), 97.69 (d), 120.2 (s), 121.6 (s), 122.3 (s), 122.5 (s), 127.0 (s), 129.1 (s), 147.1 (s), 171.8 (s),

174.4 (s) ppm.

Anal. Calcd for $C_{34}H_{56}N_2O_3$ (540.83): C, 75.51; H, 10.44; N, 5.18.
Found: C, 75.84; H, 10.50; N, 4.95.

3,17-Diethyl-1,19,21,24-tetrahydro-2,7,13,18-tetramethyl-1,19-dioxobilin-8,12-bis-eicosanoic acid [(n=19) Mesobiliverdin-XIIIα] (3). Dipyrrinone **5** (500 mg, 0.925 mmol) and *p*-chloranil (500 mg, 2.04 mmol) were dissolved in 100 mL of CH_2Cl_2. Formic acid (5 mL) was added, and the solution was heated at reflux for 12 hours. The solvent was evaporated (rotovap), and the residue was washed with 50 mL hot acetone. After cooling to room temperature the remaining solid was filtered and washed with cold acetone until the filtrate was colorless. The product was dried to afford 220 mg (45% yield) of **3**. It had mp 170°C dec.; ^{1}H-NMR (d_8-THF) δ: 1.19 (6H, t, J=7.5 Hz), 1.27 (64H, m), 1.47-1.60 (4H, m), 2.04 (6H, s), 2.18 (4H, 5, J=7.2 Hz), 2.50 (4H, q, J=7.5 Hz), 2.58 (4H, t, J=7.5 Hz), 5.88 (2H, s), 6.65 (1H, s) ppm (the NH and acid protons are not visible; at least one singlet is under the solvent signal at 1.72 ppm); ^{13}C-NMR (d_8-THF) δ: 6.92 (q), 8.11 (q), 13.30 (q), 16.85 (t), 28.71 (t), 28.92 (t), 29.06 (t), 29.20 (t), 31.06 (t), 32.84 (t), 94.34 (d), 113.4 (d), 125.9 (s), 127.9 (s), 138.7 (s), 140.3 (s), 145.2 (s), 149.3 (s), 171.5 (s), 173.1 (s) ppm (several signals overlap); FAB-MS calcd for $C_{67}H_{106}N_4O_6$ (1063.61), found 1063.9 amu.

Anal. Calcd for $C_{67}H_{106}N_4O_6$ (1063.61): C, 75.66; H, 10.05; N, 5.27.
$C_{67}H_{106}N_2O_6 \cdot$ acetone (1121.68): C, 74.96; H, 10.06; N, 4.99.
Found: C, 75.14; H, 10.05; N, 5.43.

3,17-Diethyl-1, 10, 19, 21, 23, 24-hexahydro-2, 7, 13, 18-tetramethyl-1,19-dioxobilin-8,12-bis-eicosanoic acid [(n=19) Mesobilirubin-XIIIα] (1). (n=19)-Mesobiliverdin-XIIIα (**3**) (59 mg, 0.055 mmol) was dissolved in a mixture of 10 mL of tetrahydrofuran and 10 mL of 2-propanol. Then 200 mg (5.29 mmol) of $NaBH_4$ were added, and the slurry was sonicated for 8 hours in the dark. The reaction mixture was then diluted with 200 mL of H_2O, cooled to 0°C, carefully acidified with 0.1 N HCl and extracted with 5 x 30 mL of CH_2Cl_2. The combined organic extracts were dried over anhydr. Na_2SO_4 and evaporated (rotovap). The residue was purified by flash chromatography (CH_2Cl_2-CH_3OH, 100:5 by vol.). The solvent was evaporated until a few mL were left, and methanol was then added to precipitate the product. After cooling to -30°C, filtration yielded 35 mg (59%) of the desired rubin (**1**). It had mp 125-134°C (dec.); ^{1}H-NMR (d_6-DMSO) δ: 1.07 (6H, t, J=4.2 Hz), 1.21 (64H, m), 1.45 (4H, m), 1.76 (6H, s), 1.95 (6H, s), 2.15 (4H, t, J=4.5 Hz), 3.89 (2H, s), 5.91 (2H, s), 9.80 (2H, s), 10.33 (2H, s), 11.91 (2H, s) ppm (with two signals under the solvent peak at 2.49) ppm; ^{13}C-NMR (d_6-DMSO) δ: 8.03 (q), 9.11 (q), 14.77 (q), 17.17 (t), 24.14 (t), 24.47 (t), 28.55 (t), 28.75 (t), 28.91 (t), 28.94 (t), 29.00 (t), 29.07 (t), 29.21 (t), 29.46 (t), 30.18 (t), 33.6 (t), 97.7 (d), 121.0 (s), 121.5 (s), 122.5 (s), 122.7 (s), 127.1 (s), 130.4 (s), 147.0 (s), 171.8 (s), 174.4 (s) ppm.

Anal. Calcd for $C_{67}H_{108}N_4O_6$ (1065.61): C, 75.52; H, 10.2; N, 5.26.
Found: C, 75.06; H, 10.2; N, 5.06.

Methyl 3-Ethyl-2,7,9-trimethyl-(10*H*)-dipyrrin-1-one 8-(19-oxo)eicosanoate (10). Eicosanedioic acid (596 mg, 1.79 mmol) was heated at reflux in thionyl chloride (10 mL) for 30 minutes. Excess thionyl chloride was removed at reduced pressure (rotovap), and the residue was triturated with carbon tetrachloride (2 x 5 mL). The resulting acid chloride was added to a suspension of dipyrrinone **11** (200 mg, 0.868 mmol) in 20

mL of CH_2Cl_2 and 20 mL CH_3NO_2 (100 mL three-neck, round-bottom flask with N_2-inlet and gas bubbler). Then $SnCl_4$ (904 mg, 3.47 mmol) was added, and the reaction mixture was stirred for two hours at room temperature (N_2 atmosphere). Another 904 mg (3.47 mmol) of $SnCl_4$ was added, and stirring was continued for 4.5 hours. The reaction was quenched with in methanol (20 mL), and the mixture was heated at reflux for 30 minutes. The resulting solution was poured into 300 mL of 5% aq. H_2SO_4 and extracted with CH_2Cl_2 (125 mL, then 3 x 40 mL). The combined organic extracts were washed with 125 mL of sat. aq. $NaHCO_3$ solution, dried over anhydr. Na_2SO_4 and the solvent removed under vacuum (rotovap). The solid residue was heated to reflux in 10 mL of acetone and filtered after cooling to yield 374 mg (76%) of ester **10**. The compound was used in the next step without further purification. An analytical sample was obtained by recrystallization from $CHCl_3$-CH_3OH. It had mp 119-123°C (dec.); ^{1}H-NMR, δ: 1.06-1.39 (31H, m), 1.58 (2H, m), 1.68 (2H, m), 1.95 (3H, s), 2.30 (2H, t, J=7.5 Hz), 2.40 (3H, s), 2.57 (2H, q, J=7.8 Hz), 2.71-2.76 (5H, m), 3.66 (3H, s), 6.18 (1H, s), 10.62 (1H, s), 11.26 (1H, s); ^{13}C-NMR, δ: 8.46 (q), 11.98 (q), 14.79 (q), 15.09 (q), 17.78 (t), 24.24 (t), 24.78 (t), 28.97 (t), 29.09 (t), 29.25 (t), 29.39 (t), 29.42 (t), 29.48 (t), 29.51 (t), 33.94 (t), 42.63 (t), 51.3 (q), 100.0 (d), 122.4 (s), 122.8 (s), 123.5 (s), 125.9 (s), 128.8 (s), 139.4 (s), 148.7 (s), 174.2 (s), 174.3 (s), 197.9 (s) ppm.

Anal. Calcd for $C_{35}H_{56}N_2O_4$ (568.84): C, 73.89; H, 9.93; N, 4.93.
Found: C, 73.49; H, 10.01; N, 4.97.

Methyl 3-Ethyl-2, 7, 9, trimethyl-(10*H*)dipyrrin-1-one-8-eicosanoate (7). The (19-oxo)eicosanoate **10** (890 mg, 1.56 mmol) was suspended in dry THF (130 mL) and cooled to 0°C. Sodium borohydride (120 mg, 3.17 mmol) and BF_3•Et_2O (445 mg, 3.09 mmol) were added, and the mixture was stirred for 1 hour at room temperature. Afterward, the reaction mixture was poured into 5% aq. H_2SO_4 (300 mL) and extracted with CH_2Cl_2 (5 x 30 mL). The combined organic layers were washed with 1 M aq. Na_2CO_3 solution (100 mL) and dried over anhyd. Na_2SO_4. Most of the solvent was evaporated (rotovap), and the remaining solution was heated to reflux. Methanol was added until the product started to precipitate. The mixture was cooled to -30°C, and the product was collected by filtration. The product yield was 770 mg (89%). It had mp 108-110°C; ^{1}H-NMR, δ: 1.17 (3H, t, J=7.5 Hz), 1.20-1.34 (32H, m), 1.57-1.64 (2H, m), 1.94 (3H, s), 2.12 (3H, s), 2.30 (2H, t, J=7.5 Hz), 2.34-2.39 (5H, m), 2.55 (2H, q, J=7.5 Hz), 3.66 (3H, s), 6.14 (1H, s), 10.32 (1H, s), 11.27 (1H, s) ppm; ^{13}C-NMR, δ: 8.32 (q), 9.48 (q), 11.50 (q), 14.87 (q), 17.78 (t), 24.08 (t), 24.78 (t), 28.97 (t), 29.07 (t), 29.27 (t), 29.41 (t), 29.46 (t), 29.51 (t), 30.79 (t), 33.93 (t), 51.23 (q), 101.1 (d), 121.3 (s), 121.9 (s), 122.0 (s), 124.7 (s), 126.6 (s), 131.3 (s), 148.0 (s), 173.8 (s), 174.1 (s) ppm.

Anal. Calcd for $C_{35}H_{58}N_2O_3$ (554.85): C, 75.76; H, 10.54; N, 5.05.
Found: C, 75.61; H, 10.70; N, 4.87.

Methyl 3,17-Diethyl-1, 19, 21, 24-tetrahydro-2,7,13,18-tetramethyl-1,19-dioxobilin-8, 12-bis-eicosanoate [(n=19) Mesobiliverdin-XIIIα dimethyl ester] (4). A refluxing solution of dipyrrinone methyl ester **7** (740 mg, 1.33 mmol) in CH_2Cl_2 (50 mL) was added to a solution of *p*-chloranil (688 mg, 2.80 mmol) in CH_2Cl_2 (250 mL). After five minutes, formic acid (6.36 mL) was added, and the solution was heated at reflux for 5 h. The cooled reaction mixture was washed with sat. aq. $NaHCO_3$ solution (2 x 150 mL), and the organic layer was dried over anhydr. Na_2SO_4. The solvent was evaporated (rotovap); the residue was suspended in acetone (30 mL) and filtered. The filter cake was washed with acetone until the filtrate was colorless. The solid product (**7**) was dried to give 200 mg (28%) of desired product. It had mp 103-107°C (dec.); ^{1}H-NMR,

δ: 1.24 (70H, m), 1.50-1.62 (4H, m), 1.80 (6H, s), 2.05 (6H, s), 2.29 (4H, t, J=7.5 Hz), 2.47 (4H, t, J=6.9 Hz), 2.53 (4H, q, J=6.9 Hz), 3.66 (6H, s), 5.90 (2H, s), 6.61 (1H, s), 8.40 (3H, s) ppm; ^{13}C-NMR, δ: 8.16 (q), 9.37 (q), 14.25 (q), 17.64 (t), 24.37 (t), 24.77 (t), 28.97 (t), 29.08 (t), 29.28 (t), 29.40 (t), 29.42 (t), 29.49 (t), 29.54 (t), 31.27 (t), 33.93 (t), 51.34 (q), 96.36 (d), 114.0 (d), 127.3 (s), 128.0 (s), 139.4 (s), 139.9 (s), 141.2 (s), 146.5 (s), 149.6 (s), 172.4 (s), 174.1 (s) ppm.

Anal. Calcd for $C_{69}H_{110}N_4O_6$ (1091.66): C, 75.92; H, 10.16; N, 5.13.
$C_{69}H_{110}O_6N_4$ • acetone (1149.73): C, 75.22; H, 10.17; N, 4.87.
Found: C, 75.31; H, 10.15; N, 5.06.

Methyl 3,17-Diethyl-1, 10, 19, 21, 23, 24-hexahydro-2, 7, 13, 18-tetramethyl-1, 19-dioxobilin-8, 12-bis-eicosanoate [(n=19) Mesobilirubin-XIIIα dimethyl ester] (2). (n=19) Mesobilirubin-XIIIα (**1**) (40 mg, 0.0375 mmol) was dissolved in dry THF (25 mL) and cooled to 0°C. A solution of diazomethane in ether (20 mL, prepared from 500 mg N-methyl-N-nitrosourea) was added over a period of 5 minutes, and the solution was stirred for 20 minutes at 0°C and for 1 h at room temperature. Then, the solvent was evaporated (rotovap), and the residue was purified by flash chromatography (silica gel, CH_2Cl_2-CH_3OH, 100:2 by vol.) to yield 40 mg (97%) of the desired product (**2**) as a sticky paste. It had ^{1}H-NMR, δ: 1.00 (6H, t, J=7.5 Hz), 1.25 (64H, m), 1.47 (6H, s), 1.58-1.68 (4H, m), 2.08 (6H, s), 2.27-2.33 (8H, m), 2.47-2.52 (4H, m), 3.66 (6H, s), 4.09 (2H, s), 5.92 (2H, s), 10.30 (2H, s), 10.63 (2H, s) ppm; ^{13}C-NMR, δ: 7.67 (q), 9.60 (q), 14.60 (q), 17.62 (t), 24.55 (t), 24.78 (t), 28.98 (t), 29.50 (t), 29.29 (t), 29.44 (t), 29.51 (t), 29.59 (t), 29.64 (t), 31.31 (t), 33.94 (t), 51.3 (q), 100.2 (d), 120.7 (s), 122.7 (s), 123.0 (s), 12.4 (s), 128.3 (s), 130.8 (s), 146.6 (s), 173.9 (s), 174.2 (s) ppm; FAB-MS calcd for $C_{69}H_{112}N_4O_6$ (1093.63); found 1093.9 amu.

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