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Atropisomerism in linear tetrapyrroles

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Abstract—Novel bilirubin and biliverdin congeners with propionic acids replaced by *o*-carboxyphenyl exhibit diastereomerism due to axial chirality about the carbon–carbon single bond linking the *o*-carboxyphenyl group to a pyrrole ring. Evidence for atropisomerism was found even in the monopyrrole precursor, ethyl 3,5-dimethyl-4-(*o*-carboxyphenyl)pyrrole-2-carboxylate. Like bilirubin, *o*-carboxyphenyl rubin **1a** adopts an intramolecularly hydrogen-bonded ridge-tile conformation in nonpolar solvents. In solutions containing optically active amines or human serum albumin **1a** exhibits intense bisignate exciton coupling-type induced circular dichroism for its long wavelength absorption near 400 nm.

1. Introduction

Atropisomerism has become synonymous with biphenyl or biaryl stereochemistry, where sufficiently high barriers to rotation about the interconnecting sp^2-sp^2 C–C bond may lead to isolatable stereoisomers.[1] Studies of atropisomerism about a pyrrole to phenyl sp^2-sp^2 C–C bond are few,[2] and in the following we describe one such example (the monopyrrole **4**) and show how it can be detected in a dipyrrole (**3c**) and in various new linear tetrapyrrole derivatives. Among the latter, we describe the syntheses and stereochemistry of novel bilirubin (**1**) and biliverdin (**2**) analogs whose two propionic acids are replaced by *o*-carboxyphenyls (Fig. 1).

2. Results and discussion

2.1. Synthesis

Pyrroles of the type illustrated by **4** (Fig. 1), whether with an *o*-carboethoxyphenyl, *o*-carbomethoxyphenyl or *o*-carboxyphenyl group on the pyrrole nucleus are unknown, as are variants with these same *o*-substituents located *m* or *p* on the benzene ring. In principle, they might be prepared[3] by a classical Fischer–Knorr synthesis from the appropriate 3-phenylpentane-2,4-dione, a reaction not yet reported, and a dione apparently unknown. An alternative synthesis might follow one developed by Chang and Bag[4] using a Suzuki coupling of a β-bromopyrrole with phenylboronic acid. Although *m* and *p*-carboxy (or carboalkoxy) phenylboronic acids have been described in the literature, the *o*-isomer had not. We therefore decided to reserve the pyrrole syntheses of *m* and *p*-carbo(alko)xyphenyl isomers to the Suzuki coupling method and pursue a more classical synthesis of **4** (Scheme 1). For the latter, we required 3-(*o*-carboxyphenyl)pentane-2,4-dione (**5a**), which could be produced in high yield by reaction of the sodium *o*-bromobenzoate with the sodium salt of pentane-2,4-dione.[5,6] The resulting acid (**5a**) was converted to its methyl ester (**5b**) by reaction with diazomethane, but Fischer–Knorr condensation of it with diethyl oximinomalonate using zinc in acetic acid led to only a 26% isolated yield of pyrrole methyl ester **4b**—along with substantial isocoumarin side-product, 4-acetyl-3-methyl-1*H*-2-benzopyran-1-one.[6] This mixture was separated only with considerably difficulty. The problems encountered in converting **5b** to **4b** were largely overcome by treating the acid (**5a**) under the same Fischer–Knorr pyrrole-forming condensation conditions to afford a 65% isolated yield of pyrrole acid **4a**.

Saponification of **4a** or **4b** to its diacid and condensation with 5-bromomethylene-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole[7] afforded yellow dipyrrinone **3a** in 60% yield after treatment with CH_2N_2. Attempted oxidative coupling of dipyrrinone acid **3b** gave no verdin, apparently due to interference (by proton transfer) from the free CO_2H group; however, oxidative coupling of dipyrrinone ester **3a** gave a mixture of verdins (**2**) in 84% yield. Standard verdin reduction with $NaBH_4$ gave rubin ester **1b**, which was saponified to rubin diacid **1a** in 91% isolated yield.

2.2. Characterization

The structures of monopyrroles **4**, dipyrrole **3** and tetrapyrroles **1** and **2** follow logically from the method of synthesis (see Scheme 1) and the compounds were characterized by spectroscopy, especially ^{13}C NMR, which showed the expected characteristic carbon resonances for the pyrrole units and the *o*-carboxy (or

Keywords: bilirubin; conformation; hydrogen bonding.

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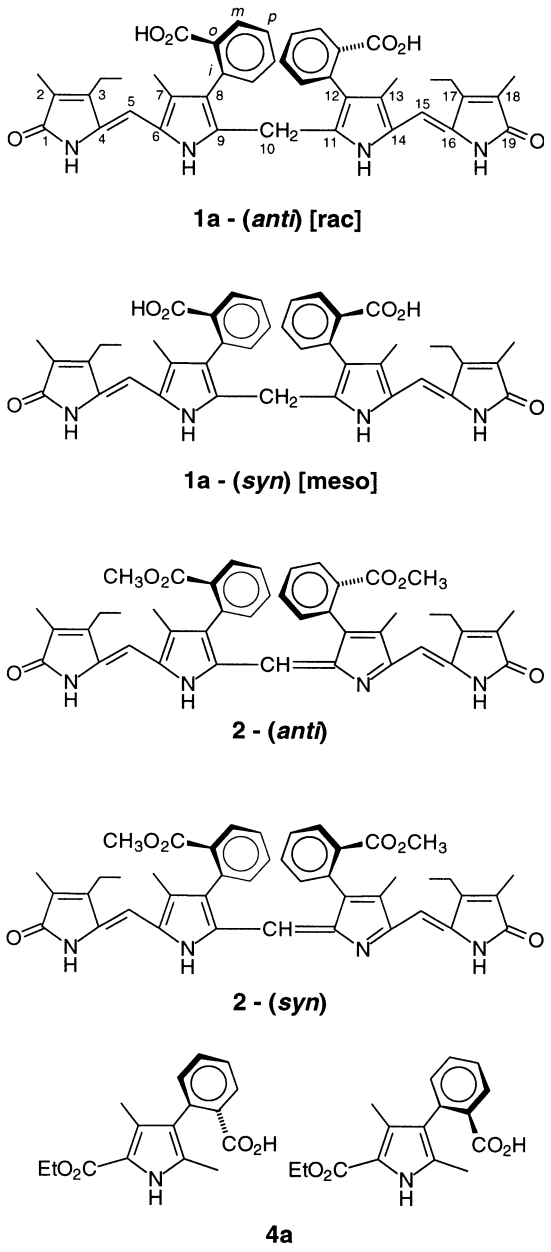
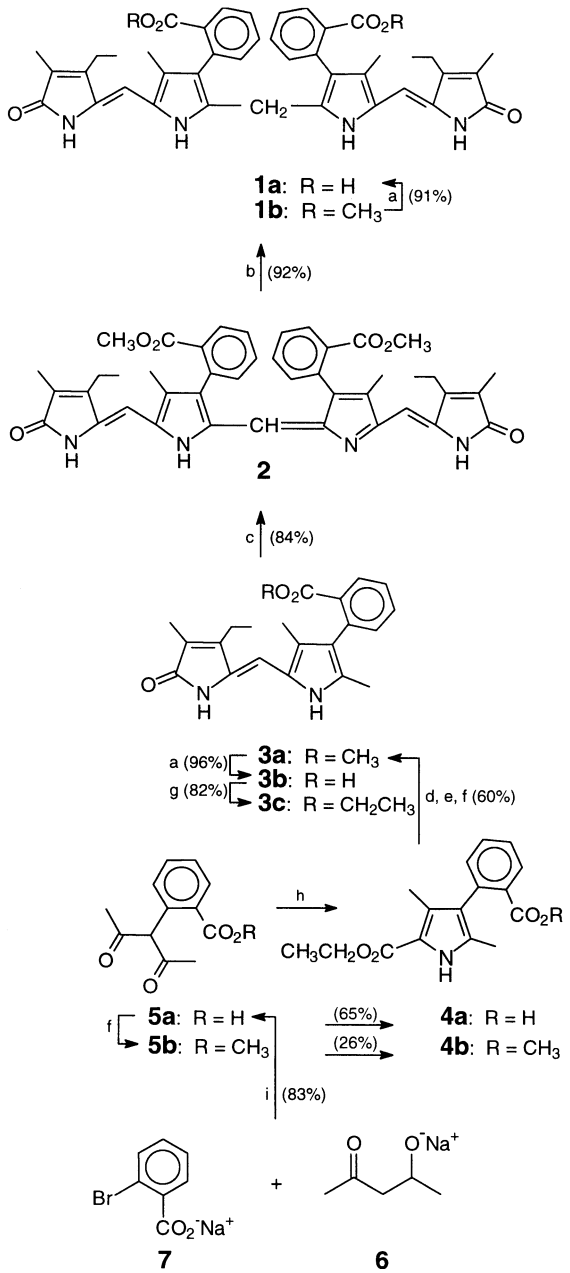


Figure 1. Atropisomeric mesobilirubins (**1**), mesobiliverdins (**2**) and monopyrroles (**4**), with restricted rotation about the pyrrole–phenyl bond. The rubins (**1**) and verdins (**2**) are shown for simplicity in the conventional linear representations.



Scheme 1. *Reagents and conditions*: (a) NaOH/EtOH–H_2O, Δ, then HCl; (b) $NaBH_4/CH_3OH$; (c) *p*-chloranil/HCOOH–CH_2Cl_2; (d) NaOH/EtOH–H_2O, Δ, then HNO_3; (e) 3-methyl-4-ethyl-5-bromomethylene-2-pyrrolinone, CH_3OH, Δ; (f) CH_2N_2; (g) CH_3CH_2OH, DCC, DMAP; (h) diethyl oximinomalonate, Zn, HOAc, NaOAc; (i) Cu_2Br_2/EtOH, Δ, then HCl.

carboalkoxy) phenyl group. In **1**–**4**, the four benzene ring hydrogens constitute an ABCD system of chemical shifts and splittings. In **4**, for example, the most deshielded hydrogen (H_D) is *ortho* to the CO_2R group, as predicted by theory and confirmed by its NOE to the $-OCH_3$ hydrogens of **4b**. The most shielded hydrogen (H_A) is *ortho* to the pyrrole ring (*meta*$^{\prime}$ to the CO_2R), as shown by an NOE between it and the pyrrole methyls at C(3) and C(5). The apparent first order coupling constants for H_D and H_A are in accord with predictions of one vicinal coupling ($^3J\sim7-8$ Hz) and one W-coupling ($^4J\sim1$ Hz) with the inner aromatic hydrogens. The assignments of the two remaining (inner) aromatic hydrogens, H_B and H_C, were made as *meta* and *para* to the CO_2R group, respectively, on the basis of an NOE seen between H_A and H_C, and between H_B and H_D. The apparent first-order coupling constants of H_B and H_C are consistent with predictions: two vicinal couplings ($^3J\sim7-8$ Hz) and one W-coupling ($^4J\sim1$ Hz).

The UV–Vis spectra of verdin **2** differed little from that of mesobiliverdin-XIIIα dimethyl ester, while those of rubin **1a** differed little from the UV–Vis spectra of mesobilirubin-XIIIα, indications that the *o*-carboxyphenyl groups do not perturb the tetrapyrrole chromophores and suggestive of an orthogonal relationship between the pyrrole ring and attached phenyl ring.

Rubin **1a** was more soluble in $CHCl_3$ than mesobilirubin-XIIIα and sufficiently soluble in $CHCl_3$ for molecular

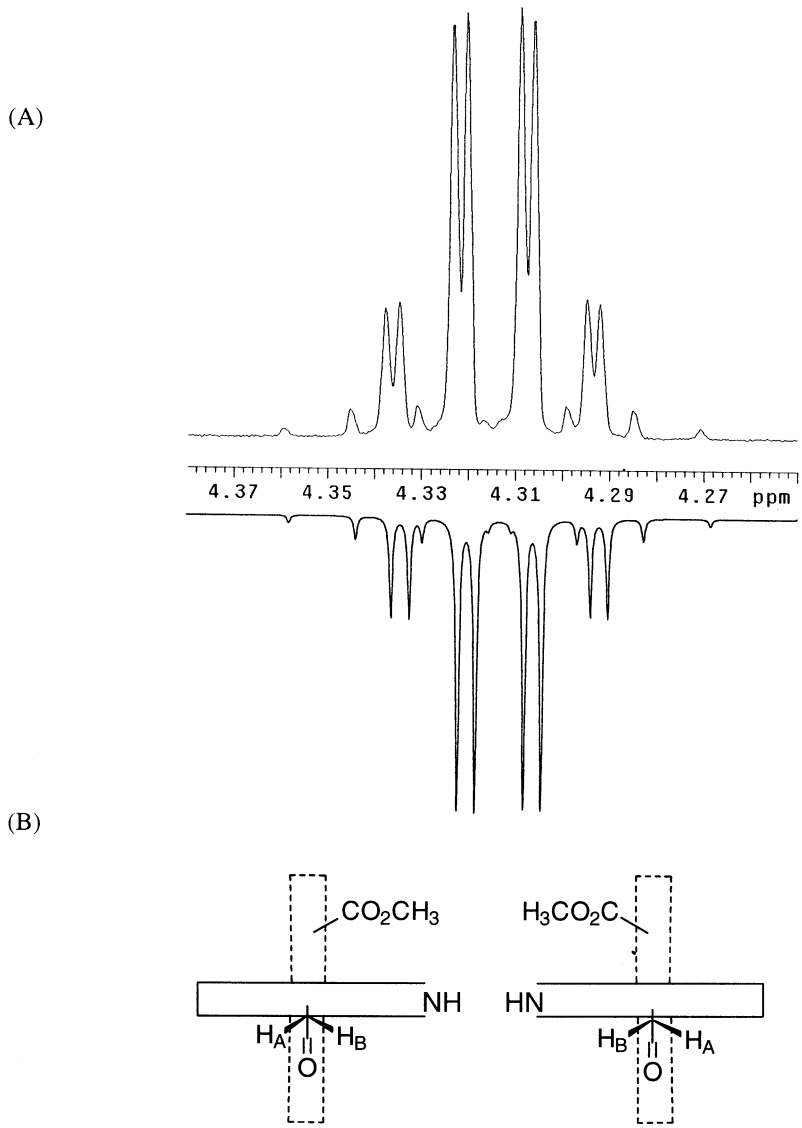


Figure 2. (A) Partial ^{1}H NMR spectrum of monopyrrole **4b** in $CDCl_3$ at 25°C showing an ABX_3 spin system for the CH_2 protons of the ethyl ester. The inverted spectrum is of the spin-simulated ABX_3 system, showing only the AB part and generated using ν_A=2154.4 Hz, ν_B=2161.1 Hz, ν_X=679.8 Hz, J_{AB}=10.9 Hz, and J_{AX}=J_{BX}=7.1 Hz. The chemical shifts ν_A and ν_B, and the coupling constant J_{AB} were determined by spin-decoupling of X_3 and were used as input simulation parameters. (B) Stereo-diagrams illustrating diastereotopic CH_AH_B hydrogens of the ethyl ester atropisomeric **4b** enantiomers.

weight determination by vapor pressure osmometry at 45°C for concentrations in the range 10^{-2}–10^{-3} M. As with most rubins capable of intramolecular hydrogen bonding, **1a** was monomeric in $CHCl_3$: measured MW 655±30 g/mol (FW 685 g/mol). Surprisingly, its dimethyl ester (**1b**) was also monomeric in $CHCl_3$: measured MW 733±20 g/mol (FW 713 g/mol). This finding is unusual, as most rubin dimethyl esters are dimeric in $CHCl_3$.[8] Apparently, the phenyls interfere with the conformation required for dimer formation.

On silica gel TLC chromatographic analysis, however, rubin ester **1b** (R_f 0.10, 1% CH_3OH in CH_2Cl_2) is more polar than rubin acid **1a** (R_f 0.54), as is typically found for rubins capable of intramolecular hydrogen bonding, e.g. mesobilirubin-XIIIα (R_f 0.77, 1% CH_3OH in CH_2Cl_2) and its dimethyl ester (R_f 0.21). Somewhat contradictorily, these chromatographic data suggest that while **1a** is most probably intramolecularly hydrogen-bonded, it is also somewhat more polar than its mesobilirubin-XIIIα analog—possibly because the large π-electron rich phenyl groups of the *o*-benzoic acids are adsorbed to the silica more strongly than the simple CH_2–CH_2– units of the propionic acid chains.

2.3. Stereochemistry

First in monopyrrole **4b**, and then in its progeny, we detected an unexpected event in the ^{1}H NMR that provided the first indication of axial chirality, that **4b** is a mixture of enantiomers. Although all proton and carbon (APT) resonances had the expected, typical chemical shifts and splitting patterns in $CDCl_3$, the methylene group of the ethyl ester did not. In the ^{1}H NMR spectrum it showed at least 14 lines, suggesting an ABX_3 spin system (Fig. 2). Selective decoupling of X_3 at 1.36 ppm, revealed an AB pattern: two different, tightly coupled resonances at 4.310 and 4.323 ppm, and ^2J=10.9 Hz. We take this as evidence for

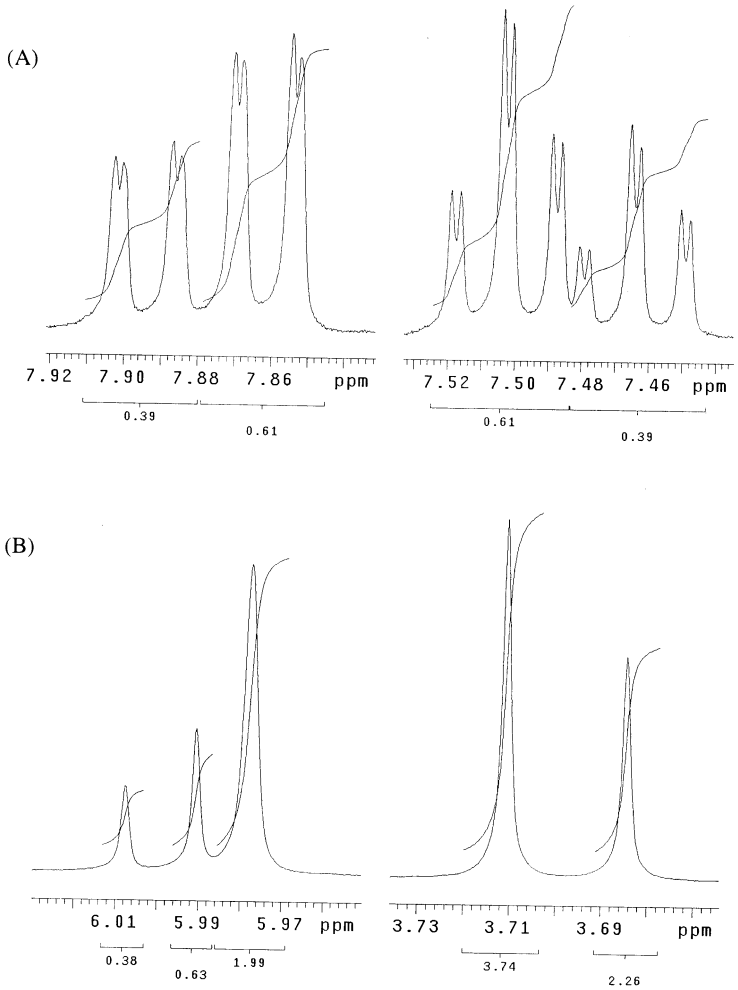


Figure 3. Partial ^{1}H NMR spectra of verdin **2** in $CDCl_3$ at 25°C showing: (A) doubled sets of signals in the aromatic region, 2:3 or 3:2 integral ratio, and (B) doubled OCH_3 ester signals, 3:2 (right) and (left) doubled C(10) methine resonances. The resonance at 5.98 is due to the C(5)/C(15) methines.

diastereotopicity and thus the presence of an axis of chirality in **4b**. Although NMR evidence for atropisomerism could be detected in **4b**, attempts to resolve the acid (**4a**) by classical methods, including crystallization of salts with alkaloids and separation of its *l*-menthyl ester derivatives, were unsuccessful.

Extensive NMR and X-ray crystallographic studies firmly established that dipyrrinones with NHs and β-alkyl groups adopt the *syn-Z* configuration.[9] This is particularly true in $CDCl_3$ solvent, where dipyrrinones form tightly intermolecularly hydrogen-bonded dimers,[9–12] but also in $(CD_3)_2SO$, where the solvent participates in hydrogen bonding to the NHs.[9] Where *E*-configuration isomers of dipyrrinones of this type have been prepared, they are unstable and revert to the more stable *Z*.[9,13–15] NOE studies of **3** are consistent with the *syn-Z* configuration. Thus NOEs are seen between the pyrrole and lactam NHs, and between the C(5) methine and the C(7) methyl and C(3)–CH_2. NOEs were not detected between the C(5)–H and the pyrrole NH (as would come from the *anti-Z* configuration), nor between the C(5)–H and the lactam NH (as would come from the *E*-configuration). For dipyrrinones **3a** and **3b**, derived from monopyrroles **4b** and **4a**, we found no evidence for diastereotopicity of the sort seen in **4b**. However, in ethyl ester **3c**, again the ethyl ester CH_2 hydrogens exhibited diastereotopicity. The chemical shift difference of the H_A and H_B resonances in **3c** is similar to that of **4b**.

Consistent with the greater stability of the *syn-Z* configuration of their component dipyrrinones, NMR NOE and crystallographic studies show that verdins and rubins adopt the favored *syn-Z* configuration.[9] The general shape of verdins is thus porphyrin-like, with a lock-washer type conformation, while that of rubins is bent into a ridge-tile shape.[9] The local stereochemistry about the pyrrole-to-phenyl bond is manifested differently in the tetrapyrroles due to an accumulation of two elements of chirality. Thus, verdin **2** can be expected to exist in at least two limiting diastereoisomeric forms, one with the *o*-CO_2CH_3 groups *syn* (relative to the average horizontal plane of the lock-washer shaped verdin) and one with them *anti* (Fig. 1). In the ^{1}H NMR spectrum of **2** one sees a doubling of the aromatic hydrogen signals (Fig. 3A) with the two sets of signals appearing in a 40:60 ratio. Doubled resonances also appear for the C(7)/C(13) methyls, the ester OCH_3 and the C(10) methine (Fig. 3B). The C(5)/C(15) methines do not, however, show a doubling (Fig. 3B, left). The

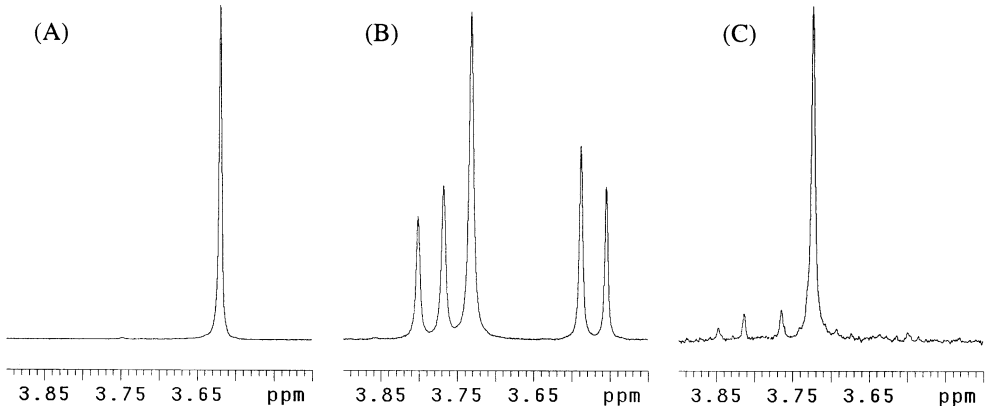


Figure 4. Partial ^{1}H NMR spectra of the C(10) CH_2 region of **1a** in (A) $CDCl_3$, (B) $(CD_3)_2SO$, (C) CD_3OD at 25°C. Only a singlet is found in (A). In (B), and to a very small extent in (C), a singlet is superimposed on a pair of doublets (65 and 12%, respectively). The singlet corresponds to C(10) CH_2 of the *anti* conformation, the *AB* pattern corresponds to the *syn*.

considerable shielding of the C(10) methine from its normal chemical shift of 6.9–7.0 ppm is probably due to π-facial shielding by the nearby phenyl rings.

Signal doubling of the type seen by ^{1}H NMR of verdin **2** is also seen in rubin ester **1b** and can be detected in diacid **1a** (Fig. 4). The C_2-symmetric rubins (**1a**-*anti*, Fig. 1, and **1b**-*anti*) have their C(10) CH_2 hydrogens in identical electronic environments and these hydrogens appear as a singlet in $CDCl_3$. In the C_S-symmetric rubins (**1a**-*syn*, Fig. 1, and **1b**-*syn*) the C(10) CH_2 hydrogens lie in different electronic environments and their resonances appear at different chemical shifts as a four-line pattern, characteristic of an AB spin system. The integral ratio of the C(10) CH_2 resonances of **1a** thus serves as a sensitive probe of conformational (atropisomerism) preference.

The ^{1}H and ^{13}C NMR of **1a** in $CDCl_3$ differed from that of **1b** and **2**, e.g. by exhibiting only a singlet for the C(10) CH_2, an indication that **1a** adopted exclusively the *anti* conformation. Consistent with this assignment, the C(7) and C(13) methyls (1.81 ppm) and C(10) CH_2 (3.62 ppm) are more shielded in **1a** than in mesobilirubin-XIIIα (2.14 and 4.06 ppm, respectively). The *anti* diastereomer (Fig. 1) of **1a** can adopt a ridge-tile conformation with both CO_2H groups hydrogen-bonded intramolecularly to dipyrrinones (Fig. 5),[16] and the dipyrrinone NH chemical shifts (9.46 ppm, pyrrole; 10.47 ppm, lactam) provide supporting evidence. (In mesobilirubin-XIIIα, known to adopt an intramolecularly hydrogen-bonded ridge-tile conformation in $CHCl_3$, the pyrrole NH resonates at 9.16 ppm, and the lactam at 10.59 ppm.) More direct evidence for hydrogen bonding and the *syn-Z* configuration of the dipyrrinones in **1a**-*anti* was found by NOE experiments. A ROESY spectrum indicated strong NOEs between the pyrrole and lactam NHs, and between the C(5)/C(15) methine and the C(3)/C(17)–CH_2CH_3 and the C(7)/C(13) methyls—all consistent with a *syn-Z* conformation in the dipyrrinones. A steady state NOE difference spectrum in $CDCl_3$ confirmed the *syn-Z* stereochemistry and also showed an NOE from the lactam NH to the *o*-carboxylic acid proton. The latter confirms that intramolecular hydrogen bonding is likely. A pulsed field gradient spin-echo NOE experiment showed that when the C(10) CH_2 protons were irradiated, NOEs were found to the phenyl ring *o′*-hydrogens as well as to the pyrrole NHs, thus strongly suggesting a rigid conformation around the pyrrole–phenyl bond in nonpolar solvents.

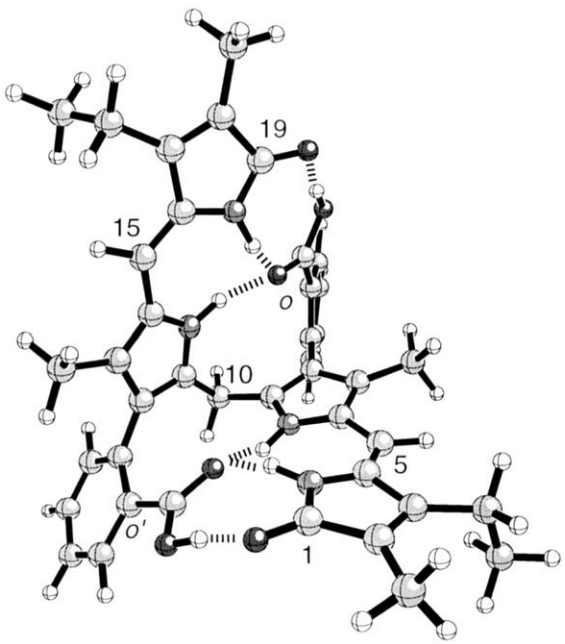


Figure 5. Ball and Stick (Ref. 16) conformational representation of the most stable conformer of **1a**, with two dipyrrinones shaped like a ridge-tile and hydrogen bonded intramolecularly to the opposing *o*-carboxyphenyl groups.

Apparently, the atropisomeric conformational homogeneity found for **1a** in $CDCl_3$ is forced by intramolecular hydrogen bonding (Fig. 5) because when this constraint is lifted, as found in polar solvents, the *syn* isomer may be detected by ^{1}H NMR (again by the C(10) CH_2 signal). In the most dramatic case, in $(CD_3)_2SO$, for example, we find a 35:65 ratio of *anti*/*syn* (Fig. 4). The same signal doubling and intensity ratio are observed in the ^{13}C NMR spectrum of **1a** in $(CD_3)_2SO$. In CD_3OD, however, the *anti* still predominates, 88:12, over the *syn*. The C(10) CH_2 resonances from **1a** in $(CD_3)_2SO$ did not change noticeably upon brief warming from 50 to 110°C, nor did they in $CDCl_2CDCl_2$ from 20 to 140°C for a prolonged time. A survey of the influence of solvents on the COOH, NH and C(10) CH_2

Table 1. Solvent influence on the carboxylic acid, pyrrole, lactam and C(10)–CH_2 ^{1}H NMR chemical shifts and *anti*/*syn* diastereomer ratio of **1a** at 25°C

Solvent	ε[a]	Acid	Lactam NH	Pyrrole NH	C(10)–CH_2		Ratio	
					anti	*syn*	*anti*	*syn*
C_6D_6	2.3	14.70	10.80	9.57	3.39		100	0
$CDCl_3$	4.7	13.60	10.47	9.46	3.62		100	0
THF-d_8	7.3	13.75	10.21	9.51	3.58		100	0
CD_2Cl_2	8.9	13.74	10.46	9.37	3.61		100	0
$CDCl_2CDCl_2$	10.4	13.45	10.19	9.30	3.54		100	0
$(CD_3)_2CO$	20.7	13.79	10.40	9.48	3.63		100	0
CD_3OD	32.6	–	–	–	3.72	3.75, 3.83	88	12
CD_3CN	36.2	–	10.35	9.30	3.54		100	0
$(CD_3)_2NCDO$	36.7	13.10	sh 9.67, 9.64	10.38, 10.28	3.83	3.80, 3.88	42	58
$(CD_3)_2SO$	46.5	12.32	9.77, 9.71	10.24, 10.08	3.73	3.58, 3.78	35	65
CD_3COOD	6.2	–	–	–	3.93	3.88, 4.04	72	28
Pyridine-d_5	12.3	12.47	sh 10.91, 10.59	10.90, 10.52	3.80	4.18, 4.31	76	24

Concentration 2×10^{-3} M.
[a] Solvent dielectric constant for protiosolvents from Gordon, A. J.; Ford, R. A. *The Chemist's Companion*; Wiley: New York, 1972; pp 4–13.

Table 2. Solvent dependence of the NH ^{1}H NMR chemical shifts and diastereomer ratio of **1b**

Solvent	Pyrrole NH	Lactam NH	Ratio[a] *anti*/*syn*
C_6D_6	*11.04*, 11.44	*11.19*, 11.68	37:63
$CDCl_3$	*8.92*, 10.85 (*9.84*, 10.97)[b]	*9.82*, 10.50 (*10.21*, 10.58)[b]	79:21
CD_3CN	10.79, *10.88*	10.53	48:52
$(CD_3)_2SO$	10.13, *10.20*	*9.72*, 9.79	46:54

Solutions 2×10^{-3} M at 25°C.
[a] Measured by the integral intensity of singlet *anti*-**1b** and AB *syn*-**1b** signals of the C(10)–CH_2 (the italicized chemical shifts dominate).
[b] Concentration 8×10^{-3} M.

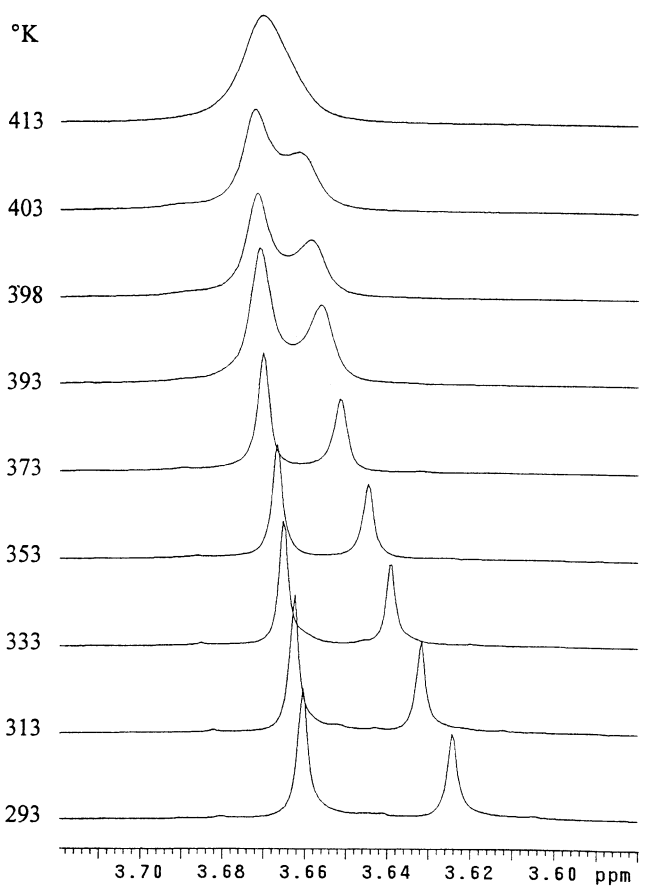


Figure 6. Variable temperature partial ^{1}H NMR spectrum of **2** in $CDCl_2CDCl_2$ solvent.

chemical shifts and the *anti*/*syn* diastereomer ratio is summarized in Table 1. Solvents that interfere with intramolecular hydrogen bonding appear to have the greatest influence in raising the relative amount of *syn* diastereomer, where at least one carboxyl group cannot engage in intramolecular hydrogen bonding and is freely solvated.

In the rubin dimethyl ester (**1b**), where intramolecular hydrogen bonding is not expected to play a dominating role, the *anti*/*syn* diastereomer ratios are also solvent dependent. Here, however, substantial amounts of *syn* diastereomer are present in aprotic solvents (Table 2), and the ratios do follow a logical progression. Curiously, whereas the NH chemical shifts of **1b**-*anti* are noticeably concentration-dependent, with the pyrrole NH being deshielded by ~0.9 ppm and the lactam NH deshielded by ~0.4 ppm following a 3–4 fold concentration increase, in **1b**-*syn* they are not affected much. Although the data might suggest an aggregation phenomenon, vapor phase osmometry studies of **1b** (and **1a**) clearly indicate that these compounds are monomeric in $CHCl_3$ in the concentration range 10^{-2}–10^{-3} M at 45°C. While it is not unusual to find that bilirubins are monomeric in $CHCl_3$, when they are capable of intramolecular hydrogen bonding it is unusual to find bilirubin esters that are not dimeric in $CHCl_3$.[8]

2.4. Rotation barrier

Variable high temperature ^{1}H NMR experiments of verdin **2** in $CDCl_2CDCl_2$ solvent showed coalescence at 413 K (Fig. 6) of the diastereotopic methyl ester CH_3 groups. Diastereotopic because **2** is a mixture of interconverting **M**

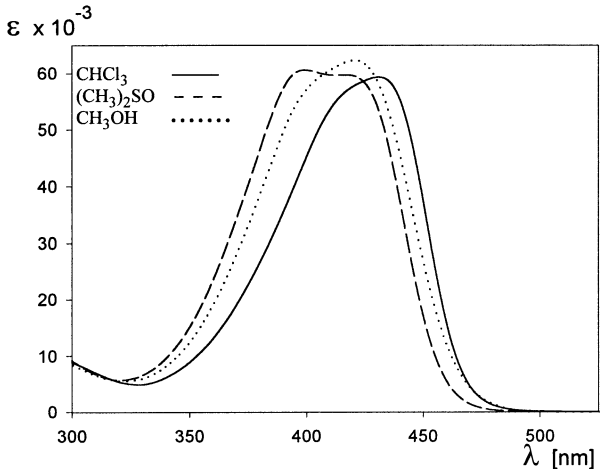


Figure 7. UV–Vis spectra of 1.60×10^{-5} M rubin **1a**. In $CHCl_3$: ε_{431}^{max} 59,300, ε_{419}^{sh} 57,400. In CH_3OH: ε_{421}^{max} 62,300, ε_{401}^{sh} 57,800. In $(CH_3)_2SO$: ε_{416}^{max} 59,700, ε_{399}^{max} 60,600.

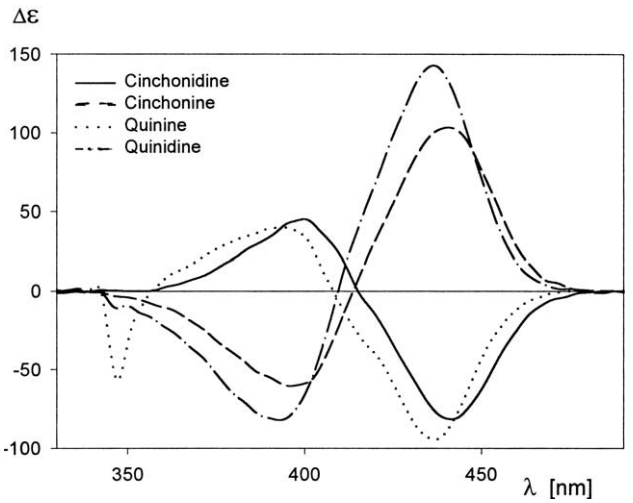


Figure 9. Induced CD of rubin **1a** in $CHCl_3$ in presence of cinchona alkaloids.

and **P** helical conformers (with a low interconversion barrier ~10 kcal/mol), each capable of atropisomerism about the pyrrole to phenyl bond axis of chirality. Analysis by variable temperature ^{1}H NMR (Fig. 6) indicates a rate constant (K_c) at coalescence temperature of ~40 s^{-1} from the equation: $K_c = \pi\Delta\nu\sqrt{2}$. Using the Eyring equation ($\Delta G^{\ddagger}=RT_c$ $(23.76-\ln(K_c/T_c))$, where K_c=40 s^{-1} and T_c=413 K, we estimate an interconversion barrier of $\Delta G^{\ddagger}$=21.4 kcal/mol for rotation about the pyrrole to phenyl bond—or too low to isolate the atropisomers.

2.5. Circular dichroism

The two dipyrrinone chromophores in bilirubins interact as an exciton system, as observed by UV–Vis spectroscopy and especially circular dichroism (CD) spectroscopy. Rubin **1a** and its dimethyl ester (**1b**) offer no exception. Thus, in $(CH_3)_2SO$, their UV–Vis spectra are dimpled, with ε_{399}^{max} 60,600, ε_{416}^{max} 59,700 for **1a** (Fig. 7) and ε_{397}^{max} 62,500, ε_{418}^{sh} 59,900 for **1b** (Fig. 8). In $CHCl_3$ and in CH_3OH, the spectra of **1a** are also dimpled (Fig. 7), with λ_{max} and ε^{max} of nearly the same values as in $(CH_3)_2SO$. The bathochromic spectral shifts observed for the solvent changes in the UV–Vis spectra of **1a** (Fig. 7) are probably associated with a change toward more complete intramolecular hydrogen bonding in $CHCl_3$ (Fig. 5).

In the presence of chiral amines (cinchona alkaloids), intense bisignate long wavelength CD Cotton effects are observed for **1a** (but not **1b**) (Fig. 9). As with bilirubin, the signed order of the Cotton effects in $CHCl_3$ correlates with a negative exciton chirality (and negative torsion angle between relevant electric dipole transition moments) when quinine or cinchonidine are present, and with a positive exciton chirality when cinchonine or quinidine are present. The induced CD magnitudes are similar to those observed for bilirubin.

In alkaline buffer, and in the presence of human serum albumin (HSA), bisignate CD Cotton effects are also observed. The exciton chirality of **1a** in the presence of HSA is the same as that observed for bilirubin, and the magnitude of the CD is also similar (Fig. 10).

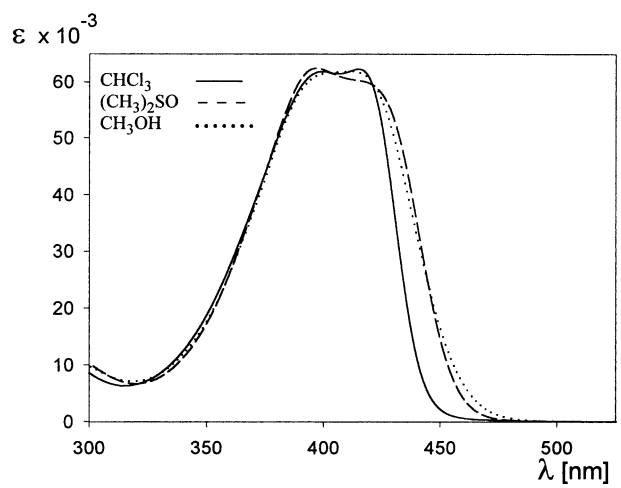


Figure 8. UV–Vis spectra of 1.89×10^{-5} M rubin dimethyl ester **1b**. In $CHCl_3$: ε_{415}^{max} 62,300, ε_{399}^{max} 61,800. In CH_3OH: ε_{411}^{max} 61,900, ε_{399}^{sh} 61,400. In $(CH_3)_2SO$: ε_{418}^{sh} 59,900, ε_{397}^{max} 62,500.

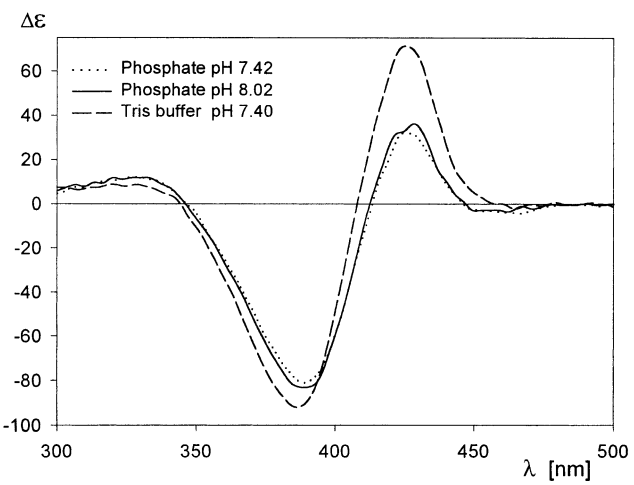


Figure 10. Induced CD of rubin **1a** from complexation with HSA in aqueous alkaline buffers.

3. Conclusions

Synthetic rubin **1a** is found to adopt preferentially a conformation shaped like a ridge-tile or half-opened book where the *o*-benzoic acids are engaged in intramolecular hydrogen bonding to the pigment's dipyrrinone components. As in natural bilirubin, the yellow pigment of jaundice, such intramolecular hydrogen bonding stabilizes the ridge-tile conformation. Unlike bilirubin the *o*-benzoic acid components present the opportunity for atropisomerism, for which a barrier of ~21 kcal/mol has been estimated by DNMR measurements.

4. Experimental

4.1. General procedures

Nuclear magnetic resonance spectra were obtained on a Varian Unity Plus spectrometer at 11.75 T magnetic field strength operating at ^{1}H frequency of 500 MHz and ^{13}C frequency of 125 MHz. $CDCl_3$ solvent was used throughout (unless otherwise specified), and chemical shifts are reported in δ ppm, referenced to the residual $CHCl_3$ ^{1}H signal at 7.26 ppm and $CDCl_3$ ^{13}C signal at 77.00 ppm. *J*-modulated spin-echo (Attached Proton Test) and gHMBC experiments were used to obtain the ^{13}C NMR assignments. The apparent multiplicities and values of spin–spin coupling constants (^{3}J, ^{4}J)[17] of all aromatic protons were confirmed by single-frequency homonuclear decoupling experiments and by the Varian simulation routine. All UV–Vis spectra were recorded on a Perkin–Elmer Lambda 12 spectrophotometer and the circular dichroism spectra were recorded on a JASCO J-600 dichrograph. Gas chromatography–mass spectrometry analyses were carried out on a Hewlett–Packard 5890A gas chromatograph (30 m DB-1 column) equipped with a Hewlett–Packard 5970 mass selective detector. HPLC analyses were carried out on a Perkin–Elmer series 410 high-pressure liquid chromatograph with a Perkin–Elmer LC-95 UV–Vis spectrophotometric detector (set at 420 nm for rubinoid compounds) equipped with a Beckman Altex ultrasphere IP 5 μm C-18 ODS column (25×0.46 cm) kept at 34°C. The flow rate was 1 mL per minute, and the mobile phase was 0.1 M di-*n*-octylamine acetate buffer in 5% H_2O in methanol (v/v) with pH 7.7 at 22°C. Radial chromatography was carried out on Merck silica gel PF_{254} with $CaSO_4$ binder preparative layer grade, using a Chromatotron (Harrison Research, Inc., Palo Alto, CA) with 1, 2, or 4 mm thick rotors and analytical thin-layer chromatography was carried out on J. T. Baker silica gel IB-F plates (125 μm layer). Melting points were determined on a Mel-Temp capillary apparatus and are uncorrected. The combustion analyses were carried out by Desert Analytics, Tucson, AZ.

4-Dimethylaminopyridine (DMAP) and dicyclohexylcarbodiimide (DCC) were from Aldrich. Di-*n*-octylamine was from Fluka. Methanol, chloroform, ethyl acetate and dichloromethane were HPLC grade from Fisher. Sodium borohydride, *p*-chloranil, sodium acetate, sodium hydroxide, anhydrous sodium sulfate, anhydrous magnesium sulfate, sodium nitrate, sodium bicarbonate, nitric acid, hydrochloric acid, 96% formic acid and ethanol were from Fisher. Argon and nitrogen were from Air Products. Solvents and reagents were used directly as provided by the vendor, except for the Fisher HPLC-grade solvents, which were further purified by standard procedures as described in detail in Ref. 18. The spectral data were obtained in spectral grade solvents, used as provided by Aldrich or by Fisher.

4.1.1. Ethyl 4-(*o*-carboxyphenyl)-3,5-dimethyl-1*H*-pyrrole-2-carboxylate (4a). To a preheated (~80°C) mixture of 9.81 g (150 mgA) of zinc, 10.3 g (125 mmol) of anhydrous sodium acetate and 40 mL of acetic acid were added simultaneously in small portions 11.01 g (50 mmol) of 3-(*o*-carboxyphenyl)-pentane-2,4-dione (**5a**)[5] and a solution of 18.92 g (100 mmol) of diethyl oximinomalonate[19] in 12 mL of acetic acid during 45 min while maintaining the temperature at 90–95°C. After the additions were complete, the mixture was heated at vigorous reflux for 4 h and then poured into 400 mL of ice and water. After stirring for 30 min, the aqueous layer was decanted, and the semisolid product was dissolved in a minimum volume of 45°C ethanol (~65 mL) and then precipitated by slow addition of ice-cold water over 1 h. After cooling for an additional hour in an ice bath, the crude product was collected by filtration, washed with water and dried. Purification of the product by radial chromatography (1.5–5.0% v/v CH_3OH in CH_2Cl_2) and recrystallization of the isolated solid from ethyl acetate–hexane afforded 9.33 g (65%) of pyrrole **4a**. It had mp 199–200°C; ^{1}H NMR: δ 1.35 (3H, t, J=7.1 Hz), 2.03 (3H, s), 2.14 (3H, s), 4.32 (1H, ABX_3, ^{3}J=7.1 Hz, ^{2}J=11.0 Hz), 4.34 (1H, ABX_3, ^{3}J=7.1 Hz, ^{2}J=11.0 Hz), 7.22 (1H, dd, ^{3}J=7.6 Hz, ^{4}J=1.0 Hz), 7.42 (1H, ddd, ^{3}J= 7.6, 7.5 Hz, ^{4}J=1.0 Hz), 7.55 (1H, ddd, ^{3}J=7.6, 7.5 Hz, ^{4}J=1.2 Hz), 8.04 (1H, dd, ^{3}J=7.6 Hz, ^{4}J=1.2 Hz), 9.50 (1H, brs), 10.34 (1H, very brs) ppm; ^{13}C NMR: δ 11.40, 11.73, 14.52, 60.11, 117.25, 123.12, 127.06, 127.33, 130.75, 130.92, 131.05, 131.98, 132.86, 135.77, 162.58, 171.22 ppm. MS *m*/*z* (rel. abund.): 287 ($M^{+\cdot}$, 100%), 270 (41%), 242 (55%), 214 (61%) amu. Anal. calcd for $C_{16}H_{17}NO_4$ (287.3): C, 66.88; H, 5.96; N, 4.88. Found: C, 66.79; H, 5.78; N, 4.79.

4.1.2. Ethyl 4-(*o*-methoxycarbonylphenyl)-3,5-dimethyl-1*H*-pyrrole-2-carboxylate (4b). Methyl ester **4b** was obtained by the same procedure as for **4a** by using 3-(*o*-methoxycarbonylphenyl)pentane-2,4-dione (**2**)[5]—except that after separating the crude product, it was dissolved in 150 mL of CH_2Cl_2 and treated with 100 mL of 1 M aq NaOH with vigorous stirring for 6 h. The organic layer was washed with water, dried (anh. $MgSO_4$), and filtered. The solvent was evaporated under vacuum. Radial chromatography of the residue (hexane–ethyl acetate, gradient 5.5:1 to 2.5:1 v/v) followed by recrystallization of the isolated solid from ethanol–water afforded 3.95 g (26%) of pyrrole **4b**. It had mp 135–136°C; ^{1}H NMR: δ 1.36 (3H, t, J=7.1 Hz), 2.09 (3H, s), 2.10 (3H, s), 3.72 (3H, s), 4.30 (1H, ABX_3, ^{3}J=7.1 Hz, ^{2}J=10.9 Hz), 4.32 (1H, ABX_3, ^{3}J= 7.1 Hz, ^{2}J=10.9 Hz), 7.21 (1H, dd, ^{3}J=7.6 Hz, ^{4}J=1.2 Hz), 7.39 (1H, ddd, ^{3}J=7.6, 7.4 Hz, ^{4}J=1.2 Hz), 7.52 (1H, ddd, ^{3}J=7.4, 7.6 Hz, ^{4}J=1.3 Hz), 7.90 (1H, dd, ^{3}J=7.6 Hz, ^{4}J= 1.3 Hz), 8.69 (1H, brs) ppm; ^{13}C NMR: δ 11.15, 11.84, 14.58, 52.02, 59.70, 117.13, 123.74, 126.91, 127.05, 129.69, 129.91, 131.31, 132.06, 132.67, 135.58, 161.81,

168.25 ppm. MS *m/z* (rel. abund.): 301 ($M^{+\cdot}$, 100%), 270 (8%), 256 (10%), 223 (83%) amu. Anal. calcd for $C_{17}H_{19}NO_4$ (301.3): C, 67.76; H, 6.36; N, 4.65. Found: C, 67.65; H, 6.38; N, 4.74.

4.1.3. 3-Ethyl-8-(*o*-methoxycarbonylphenyl)-2,7,9-trimethyl-1,10-dihydro-11*H*-dipyrrin-1-one (3a). A mixture of 4.31 g (15.0 mmol) of monoester **4a** (or 15.0 mmol of diester **4b**), 6.00 g (150 mmol) of sodium hydroxide, 90 mL of ethanol, and 25 mL of water was heated at reflux for 4 h. After cooling, the ethanol solvent was evaporated under vacuum, and the residue was diluted with 25 mL of 50% aq $NaNO_3$. The mixture was cooled to −20°C and slowly acidified with a solution of conc HNO_3 in 50% aq $NaNO_3$ (1:5 v/v). The product was separated by filtration, washed with cold water and dried overnight under vacuum. This crude diacid (obtained in a quantitative yield) was used immediately in the following step without further characterization.

A mixture of the diacid from above, 3.46 g (16.0 mmol) of 5-bromomethylene-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole,[7] 100 mL of anhydrous methanol and 1 drop of conc H_2SO_4 was heated at reflux for 10 h. Then approximately 35 mL of CH_3OH were removed by distillation, and the remaining solution was chilled overnight at −20°C. The product was separated by filtration, suspended in 100 mL of CH_3OH and 45 mL of $CHCl_3$ and treated for 10 min with ethereal diazomethane (generated from 100 mmol of *N*-nitroso-*N*-methylurea). Excess CH_2N_2 was destroyed with acetic acid. Then the solvents were evaporated under vacuum, and the residue was purified by radial chromatography (2–3% CH_3OH in CH_2Cl_2 v/v). Recrystallization of the isolated solid from CH_2Cl_2–CH_3OH afforded 3.29 g (60%) of dipyrrinone **3a**. It had mp 289–291°C (decomp); ^{1}H NMR: δ 1.19 (3H, t, J=7.6 Hz), 1.95 (3H, s), 2.01 (3H, s), 2.31 (3H, s), 2.56 (2H, q, J=7.6 Hz), 3.73 (3H, s), 6.20 (1H, s), 7.26 (1H, dd, ^3J=7.6 Hz, ^4J=0.9 Hz), 7.38 (1H, ddd, ^3J=7.6, 7.4 Hz, ^4J=0.9 Hz), 7.52 (1H, ddd, ^3J=7.6, 7.4 Hz, ^4J=1.1 Hz), 7.89 (1H, dd, ^3J=7.6 Hz, ^4J=1.1 Hz), 10.57 (1H, brs), 11.38 (1H, brs) ppm; ^{13}C NMR: δ 8.53, 10.11, 12.06, 15.03, 17.96, 52.07, 101.47, 122.46, 122.52, 122.90, 124.87, 126.56, 127.32, 129.80, 131.16, 131.83, 132.14, 132.59, 136.30, 148.37, 168.67, 174.16 ppm. Anal. calcd for $C_{22}H_{24}N_2O_3$ (364.4): C, 72.50; H, 6.64; N, 7.69. Found: C, 72.16; H, 6.41; N, 7.63.

4.1.4. 3-Ethyl-8-(*o*-ethoxycarbonylphenyl)-2,7,9-trimethyl-1,10-dihydro-11*H*-dipyrrin-1-one (3c). A mixture of 729 mg (2.00 mmol) of methyl ester **3a**, 30 mL of 10% aq NaOH, and 50 mL of ethanol was heated at vigorous reflux for 4.5 h. The ethanol solvent was removed by distillation, and the residue was cooled in an ice bath. Enough 10% aq HCl was added slowly to bring pH to <3. After stirring for 15 min, the crude dipyrrinone acid was separated by filtration, washed with water (3×50 mL) and dried under vacuum to afford 670 mg (96%) of **3b**, which did not melt but began to decompose at temperatures ≥190°C). It had ^{1}H NMR ($(CD_3)_2SO$): δ 1.09 (3H, t, J=7.6 Hz), 1.79 (3H, s), 1.90 (3H, s), 2.06 (3H, s), 2.51 (2H, q, J=7.6 Hz), 5.96 (1H, s), 7.18 (1H, d, ^3J=7.6 Hz), 7.36 (1H, t, ^3J=7.6 Hz), 7.50 (1H, t, ^3J=7.6 Hz), 7.73 (1H, d, ^3J=7.6 Hz), 9.84 (1H, s), 10.53 (1H, s), 12.57 (1H, brs) ppm; ^{13}C NMR ($(CD_3)_2SO$): δ 8.08, 9.96, 11.64, 14.85, 17.16, 97.62, 121.86, 122.16, 122.40, 122.84, 126.35, 127.68, 129.04, 129.54, 130.43, 132.07, 133.91, 134.90, 147.23 ppm.

To a mixture of 350 mg (1.00 mmol) of crude acid **3b** from above, 4 mL of anh CH_2Cl_2, 248 mg (1.20 mmol) of DCC, and 12.2 mg (0.1 mmol) of DMAP was added 175 μL (3.00 mmol) of anhydrous ethanol, and the mixture was stirred for 16 h. The solid by-product was separated by filtration. The filtrate was diluted with 100 mL of CH_2Cl_2 and washed consecutively with 5% aq $NaHCO_3$ (100 mL), 5% aq HCl (50 mL) and water (2×50 mL). After drying (anh Na_2SO_4), filtration and evaporation, the residue was purified by radial chromatography (2–3% CH_3OH in CH_2Cl_2 v/v). Recrystallization of the pure solid fractions from CH_2Cl_2–CH_3OH afforded 309 mg (82%) of dipyrrinone ethyl ester **3c**. It had mp 246–247°C; ^{1}H NMR: δ 1.13 (3H, t, J=7.0 Hz), 1.19 (3H, t, J=7.7 Hz), 1.95 (3H, s), 2.00 (3H, s), 2.30 (3H, s), 2.56 (2H, q, J=7.7 Hz), 4.15 (1H, ABX$_3$, ^3J=7.0 Hz), 4.16 (1H, ABX$_3$, ^3J=7.0 Hz), 6.19 (1H, s), 7.26 (1H, dd, ^3J=7.5 Hz, ^4J=1.0 Hz), 7.38 (1H, ddd, ^3J=7.5, 7.6 Hz, ^4J=1.0 Hz), 7.51 (1H, ddd, ^3J=7.5, 7.6 Hz, ^4J=1.3 Hz), 7.88 (1H, dd, ^3J=7.5 Hz, ^4J=1.3 Hz), 10.58 (1H, brs), 11.40 (1H, brs) ppm; ^{13}C NMR: δ 8.51, 10.10, 12.03, 13.92, 15.04, 17.97, 60.74, 101.41, 122.43, 122.50, 123.19, 125.01, 126.65, 127.34, 129.64, 131.00, 131.80, 132.44, 132.82, 136.10, 148.36, 168.50, 174.14 ppm. Anal. calcd for $C_{23}H_{26}N_2O_3$ (378.5): C, 72.99; H, 6.93; N, 7.40. Found: C, 72.64; H, 6.77; N, 7.39.

4.1.5. 3,17-Diethyl-8,12-bis-(*o*-methoxycarbonylphenyl)-2,7,13,18-tetramethyl-(21*H*,24*H*)-bilin-1,19-dione (2). A mixture of 729 mg (2.00 mmol) of dipyrrinone **3a**, 1.23 g (5.00 mmol) of *p*-chloranil, 440 mL of CH_2Cl_2, and 22 mL of 96% formic acid was heated at reflux for 24 h. The volume of the mixture was reduced to one-half by distillation, and reflux was continued for 6 h. Then the mixture was chilled overnight at −20°C. A solid separated and was removed by filtration and discarded. The blue filtrate was carefully neutralized with 5% aq $NaHCO_3$ until effervescence ceased then washed with 4% aq NaOH (2×100 mL), H_2O (4×100 mL), and dried (anh Na_2SO_4). After filtration and evaporation of the solvent under vacuum, the crude product was purified by radial chromatography (gradient CH_2Cl_2–CH_3CO_2H–CH_3OH=100:3:3 to 100:3:7 v/v/v). The combined pure fractions were washed with 1% aq $NaHCO_3$ and H_2O, then dried (anh Na_2SO_4). After filtration, the solution was evaporated and the residual was recrystallized from $CHCl_3$–hexane to afford 594 mg (84%) of *syn* and *anti* mesobiliverdins (**2**). The solid had mp 284–288°C; ^{1}H NMR: δ 1.25 (6H, t, J=7.7 Hz), 1.86 (6H, s), 1.92 (2.4H, s), 1.95 (3.6H, s), 2.54 (4H, q, J=7.7 Hz), 3.68 (2.4H, s), 3.71 (3.6H, s), 5.98 (2H, s), 5.99 (0.6H, s), 6.01 (0.4H, s), 7.15 (0.8H, dd, ^3J=7.8 Hz, ^4J=1.0 Hz), 7.17 (1.2H, dd, ^3J=7.8 Hz, ^4J=1.0 Hz), 7.36 (2H, ddd, ^3J=7.5, 7.6 Hz, ^4J=1.0 Hz), 7.47 (0.8H, ddd, ^3J=7.5, 7.8 Hz, ^4J=1.3 Hz), 7.50 (1.2H, ddd, ^3J=7.6, 7.8 Hz, ^4J=1.3 Hz), 7.86 (1.2H, dd, ^3J=7.6 Hz, ^4J=1.3 Hz), 7.89 (0.8H, dd, ^3J=7.6 Hz, ^4J=1.3 Hz), 8.29 (2H, brs), 8.75 (1H, very brs) ppm; ^{13}C NMR: δ 8.36, 9.94, 9.95, 14.42, 17.85, 52.18, 52.22, 96.29, 117.30, 117.50, 127.59, 127.61, 128.14, 128.39, 128.53, 128.55, 130.16, 130.36, 131.19, 131.42, 131.48, 131.57, 131.94, 132.12, 133.94, 134.19, 139.83,

139.86, 140.21, 141.78, 141.86, 146.68, 149.40, 149.54, 167.64, 168.01, 172.57, 172.59 ppm. Anal. calcd for $C_{43}H_{42}N_4O_6$ (710.8): C, 72.66; H, 5.96; N, 7.88. Found: C, 72.60; H, 5.99; N, 7.92.

4.1.6. 3,17-Diethyl-8,12-bis-(*o*-methoxycarbonylphenyl)-2,7,13,18-tetramethyl-(10*H*,21*H*,23*H*,24*H*)-bilin-1,19-dione (1b). To a blue solution of 355 mg (0.500 mmol) of mesobiliverdins **2** in 8 mL of $CHCl_3$ and 75 mL of anhydrous CH_3OH kept at 10°C was slowly added sodium borohydride (1.13 g, 30.0 mmol) during 20 min. while purging the mixture with N_2. After stirring for 15 min, the yellow mixture was diluted with 300 mL of ice-cold water, slowly acidified by addition of 8 mL of acetic acid followed by 6 mL of 10% aq HCl. The product was extracted with $CHCl_3$ (4×100 mL). The combined extracts were washed with water until neutral, and then dried (anh Na_2SO_4). After filtration, the solvent was evaporated under vacuum, and the residue was purified by radial chromatography (gradient 1–3% CH_3OH in CH_2Cl_2 v/v) and recrystallization from CH_2Cl_2–CH_3OH to afford 327 mg (92%) of mesobilirubin dimethyl esters (**1b**). The solid had mp 268–271°C (decomp >250°C); ^{1}H NMR (in $CDCl_3$ solvent ratio *anti*/*syn*=79:21): δ 1.05 (1.2H, t, J=7.6 Hz), 1.13 (4.8H, t, J=7.6 Hz), 1.61 (1.2H, s), 1.80 (4.8H, s), 1.85 (4.8H, s), 1.89 (1.2H, s), 2.38 (0.8H, q, J=7.6 Hz), 2.46 (3.2H, q, J=7.6 Hz), 3.66 (1.2H, s), 3.95 (4.8H, s), 3.73 (1.6H, s), 3.74, 3.95 (0.4H, AB, ^2J=15.8 Hz), 5.97 (1.6H, s), 5.99 (0.4H, s), 6.98–7.11 (2H, 2×m), 7.33–7.41 (4H, m), 7.72–7.74 (0.4H, m), 7.80 (1.6H, dd, ^3J=7.7 Hz, ^4J=1.1 Hz), 8.92 (1.6H, brs), 9.82 (1.6H, brs), 10.50 (0.4H, brs), 10.85 (0.4H, brs) ppm; ^{1}H NMR (in $(CD_3)_2SO$ solvent ratio *anti*/*syn*=46:54): δ 1.09 (3H, t, J=7.6 Hz), 1.10 (3H, t, J=7.6 Hz), 1.71 (3H, s), 1.72 (3H, s), 1.78 (3H, s), 1.79 (3H, s), 2.51 (2×2H, 2×q, J=7.6 Hz), 3.51 (3H, s), 3.54 (3H, s), 3.56, 3.75 (1H, AB, ^2J=16.9 Hz), 3.67 (1H, s), 5.91 (1H, s), 5.92 (1H, s), 6.72 (1H, dd, ^3J=7.5 Hz, ^4J=1.1 Hz), 6.78 (1H, dd, ^3J=7.6 Hz, ^4J=1.1 Hz), 7.26–7.33 (3H, m), 7.37 (1H, ddd, ^3J=7.5, 7.5 Hz, ^4J=1.5 Hz), 7.67 (1H, dd, ^3J=7.5 Hz, ^4J=1.5 Hz), 7.69 (1H, dd, ^3J=7.7 Hz, ^4J=1.5 Hz), 9.72 (1.1H, s), 9.79 (0.9H, s), 10.13 (0.9H, s), 10.20 (1.1H, s) ppm; ^{13}C NMR (in $CDCl_3$ solvent the shifts of dominant *anti* isomer are italicized): δ 7.97, *8.13*, *9.86*, 10.14, *14.73*, 14.78, 17.80, *17.83*, *23.42*, 23.72, *51.84*, 52.78, *99.55*, 100.63, 122.77, 122.95, *123.00*, 123.40, 123.45, *123.75*, *123.87*, 126.28, *126.65*, 128.74, *129.00*, *129.35*, 129.66, *130.90*, *131.07*, 131.08, 131.11, 131.70, *132.44*, *132.60*, 132.94, *135.60*, 136.00, 147.13, *147.43*, 168.21, *169.57*, *173.75*, 174.31 ppm; ^{13}C NMR (in $(CD_3)_2SO$ solvent): δ 8.07, 8.08, 9.68, 9.70, 14.85, 14.86, 17.15, 17.16, 23.76, 23.99, 51.68, 51.72, 97.69, 121.77, 121.85, 121.97, 122.13, 122.15, 122.27, 123.13, 123.17, 126.24, 126.38, 128.30, 128.45, 128.97, 129.14, 129.88, 130.33, 130.73, 131.00, 131.21, 131.35, 132.17, 135.15, 135.33, 147.12, 147.22, 167.36, 167.45, 171.96, 171.98 ppm. Anal. calcd for $C_{43}H_{44}N_4O_6$ (712.8): C, 72.45; H, 6.22; N, 7.86. Found: C, 72.15; H, 6.33; N, 7.84.

4.1.7. 8,12-bis-(*o*-Carboxyphenyl)-3,17-diethyl-2,7,13,18-tetramethyl-(10*H*,21*H*,23*H*,24*H*)-bilin-1,19-dione (1a). A solution of 285 mg (0.400 mmol) of mesobilirubin dimethyl esters (**1b**) and 30 mL of ethanol was saturated with Ar for 20 min. Then 6 mL of 1 M aq NaOH (6 mmol) was added and the mixture was heated at reflux for 90 min under Ar. After cooling, the mixture was diluted with 150 mL of H_2O and 150 mL of $CHCl_3$ then poured into 150 mL of 1% aq HCl. The product was extracted with $CHCl_3$ (4×50 mL), washed with H_2O (4×100 mL) and dried (anh Na_2SO_4). After filtration, the solvent was evaporated under vacuum, and the residue was purified by radial chromatography (gradient 1–2% CH_3OH in CH_2Cl_2 v/v) and recrystallization to afford 248 mg (91%) of bright yellow *syn* and *anti* mesobilirubins (**1a**). The solid had mp 333–338°C (decomp >305°C); ^{1}H NMR (in $CDCl_3$ solvent—exclusively *anti*): δ 1.14 (6H, t, J=7.6 Hz), 1.81 (6H, s), 1.91 (6H, s), 2.49 (4H, q, J=7.6 Hz), 3.62 (2H, s), 6.08 (2H, s), 7.18 (2H, dd, ^3J=7.5 Hz, ^4J=1.0 Hz), 7.47 (2H, ddd, ^3J=7.5, 7.8 Hz, ^4J=1.0 Hz), 7.56 (2H, ddd, ^3J=7.5, 7.5 Hz, ^4J=1.5 Hz), 8.29 (2H, dd, ^3J=7.8 Hz, ^4J=1.5 Hz), 9.46 (2H, s), 10.47 (2H, s), 13.60 (2H, brs) ppm; ^{1}H NMR (in $(CD_3)_2SO$ solvent ratio *anti*/*syn*=35:65): δ 1.09 (2.1H, t, J=7.6 Hz), 1.10 (3.9H, t, J=7.6 Hz), 1.73 (3.9H, s), 1.77 (2.1H, s), 1.78 (2.1H, s), 1.79 (3.9H, s), 2.51 (2×2H, 2×q, J=7.6 Hz), 3.57, 3.78 (1.3H, AB, ^2J=16.8 Hz), 3.74 (0.7H, s), 5.91 (0.7H, s), 5.93 (1.3H, s), 6.63 (1.3H, dd, ^3J=7.0 Hz, ^4J=1.0 Hz), 6.82 (0.7H, dd, ^3J=7.0 Hz, ^4J=1.0 Hz), 7.23–7.31 (2×2H, 2×m), 7.66 (1.3H, dd, ^3J=7.5 Hz, ^4J=1.4 Hz), 7.70 (0.7H, dd, ^3J=7.5 Hz, ^4J=1.4 Hz), 9.71 (0.7H, s), 9.77 (1.3H, s), 10.08 (0.7H, s), 10.24 (1.3H, s), 12.32 (2H, s) ppm; ^{13}C NMR (in $CDCl_3$ solvent): δ 8.03, 9.86, 14.84, 17.86, 24.20, 100.89, 123.56, 123.88, 124.17, 124.68, 127.75, 128.78, 129.91, 131.52, 131.53, 132.73, 133.73, 138.51, 148.54, 171.92, 174.92 ppm; ^{13}C NMR (in $(CD_3)_2SO$ solvent the shifts of dominant *syn* are italicized): δ 8.08, *9.86*, 9.89, 14.87, *17.17*, 17.18, *23.91*, 24.16, 97.77, *97.86*, 122.02, *122.05*, 122.10, *122.15*, *122.60*, 122.70, *123.06*, 123.11, *126.18*, 126.24, *128.24*, 128.41, *128.82*, 129.04, 130.06, *130.20*, 130.43, *130.46*, 132.02, *132.08*, 132.89, *132.96*, *134.80*, 134.89, 147.09, *147.21*, *168.94*, 169.07, 171.96, *171.97* ppm. Anal. calcd for $C_{41}H_{40}N_4O_6$ (684.8): C, 71.91; H, 5.89; N, 8.18. Found: C, 72.06; H, 5.85; N, 8.14.

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