Published in final edited form as:

Monatsh Chem. 2014 June ; 145(6): 963–981. doi:10.1007/s00706-014-1191-z.

Homorubins and Homoverdins

William P. Pfeiffer,

CIMA Labs, Inc., Eden Prairie, MN 55344 USA

Sanjeev K. Dey,

Milliken & Company, P. O. Box 1926, Spartanburg, SC 29304 USA

Heinz Falk, and

Institut für Organische Chemie, Abteilung für Organische Chemie, Johannes Kepler-Universität Linz, Altenbergerstr. 69, A-4040 Linz, Austria

David A. Lightner

Department of Chemistry, University of Nevada, Reno, Nevada, 89557-0216 USA

David A. Lightner: lightner@unr.edu

Abstract

The syntheses are described for centrally expanded bilirubin analogs: *b*-homorubins with propionic and butyric acid groups in the positions corresponding to the propionic acids of bilirubin. Their syntheses were accomplished by coupling two equivalents of a reactive monopyrrole (5-(bromomethylene)pyrrolin-2-one) to a dipyrrolethane. The corresponding *b*-homoverdins and dehydro-*b*-homoverdins were prepared by dehydrogenating the rubins or their dimethyl esters using DDQ. As supported by NMR measurements and molecular mechanics calculations, the homorubins are found to engage in conformation-determining intramolecular hydrogen bonding between the dipyrinone and carboxylic acid moieties. Likewise, the homoverdins are believed to favor intramolecularly hydrogen-bonded conformations.

Keywords

Bilirubinoids; Synthesis; Molecular Structure; Conformational Analysis

Introduction

Bilirubin (Fig. 1A), the end-product of porphyrin metabolism and the yellow pigment of jaundice [1], is capable of rotating its two dipyrinone chromophores independently about C(10) so as to bring each dipyrinone into hydrogen bonding with one of its two propionic acid groups (Fig. 1B) [2]. This conformation is required to assume a folded or half-open book shape (Fig. 1C), named “ridge-tile” [3], which minimizes non-bonded steric destabilizing interactions and found in the crystal [3–5] and solution [6–9]. It is more stable than all others, and as such it plays a dominating role in the pigment’s physico-chemical properties and metabolism [1, 10–14]. Analogs of bilirubin with vinyl groups reduced to

ethyls, e.g., mesobilirubin-XIII α (Fig. 1D) also adopt an intramolecularly hydrogen-bonded ridge-tile [2, 15] and thus exhibit similar solution and metabolic properties. In order to learn whether the ridge-tile conformation might be perturbed, yet remain stable, by linking the two dipyrinones to not one but two CH₂ connector groups, earlier we communicated [16] our synthesis of the centrally homologated mesobilirubin, 10a-homorubin, or more simply homorubin (**1**, Fig. 1E) and compared its properties to those of mesobilirubin-XIII α . This work indicated the presence of bilirubin-like intramolecular hydrogen bonding in **1**, and metabolism studies by the late Prof. A.F. McDonagh (University of California San Francisco) showed that the pigment, like bilirubin and mesobilirubin, is excreted as mono- and diglucuronides in the Sprague-Dawley rat. It was also found to be excreted unchanged, albeit slowly, in the Gunn rat (which has a congenital deficiency of the glucuronosyl transferase enzyme) and thus “appears to be an intermediate type of compound which is polar enough to be excreted without conjugation in the Gunn rat yet capable of being glucuronidated.”

Since our first communication on homorubin, a complete conformational analysis of **1** has been achieved, and the study of homorubins (*b*-homorubins) has been extended to a synthesis and analysis of the butyric acid homolog **2** (Fig. 1). Interest in **2** stems from previous studies of bilirubin analogs with propionic acids replaced by butyric acids that showed the pigment retained complete intramolecular hydrogen bonding, adopted a more open ridge-tile conformation, but still retained many of the mesobilirubin-like solution properties [17, 18].

Like bilirubin and mesobilirubin, both homorubins **1** and **2** were oxidized to the corresponding “verdins”. As noted earlier by Chen et al. [19] there are two possible verdin types: 10,10a-dehydro-10a-homorubin (*b*-homoverdin), as in **3** and **4** (Fig. 1G), and 10,10a, 22,23-didehydro-10a-homorubin (dehydro-*b*-homoverdin), as in **5** and **6** (Fig. 1H). In our work, the corresponding dimethyl esters would be labeled **3e** and **4e**, and **5e** and **6e**, which were prepared along with **3-5**. Chen et al. [19] prepared a homoverdin dimethyl ester by an entirely different method involving “2 + 2” coupling and characterized it as **3e**. From the corresponding homorubin possessing all methyl substituents, a dehydro-*b*-homoverdin with all methyl substituents at the pyrrole/pyrrolinone β -positions was also prepared by Chen and Falk [20], an analog of **5e**. Considerations of double bond stereochemistry and conformational analysis of the homoverdin diacids **2-6** indicates possible intramolecularly hydrogen-bonded conformations. Just as with the homorubins, analysis of the homoverdin structures indicates new and different hydrogen-bonded conformations of varying shape.

In the following, we report on the syntheses and conformational analysis of the homorubins and homoverdins of Fig. 1 and discuss their structures and stable conformations.

Results and Discussion

Homorubin synthesis aspects

To achieve the syntheses of **1** and **2**, we conceived of two possible logical routes to the skeletal framework (Scheme 1): “2 + 2” and “1 + 2 + 1” [21]. In the first, a dipyrinone with a 9-CHO group would be self-coupled by Ti⁰ in the McMurry reaction [22]. In the second,

two equivalents of (bromomethylene)pyrrolinone would be condensed with a α,α' -dipyrrolethylene prepared by reduction of the α,α' -dipyrrolethene produced by Ti^0 assisted self-condensation of a pyrrole α -aldehyde. Our attempts to self-condense an appropriate dipyrnone α -aldehyde ("2 + 2") proved fruitless using Ti^0 [22, 23], doubtless in part due to the insolubility of the reactant pigment and possibly adventitious reaction of the pigment with the titanium. Consequently, this approach was abandoned in favor of what became the successful "1 + 2 + 1" route diagrammed in Scheme 1.

The syntheses of **1** and **2** thus followed a straightforward pattern (Scheme 2) whereby the end ring pyrrolinone precursor, 5-(bromomethylene)-4-ethyl-3-methyl-2-oxo-2,5-dihydropyrrole [24], was condensed [16, 17, 24, 25] by HBr catalysis in hot CH_3OH with a suitable 1,2-dipyrrolethane (**13** and **14**). Initially, we thought that condensation using ethenes **11** or **12** might suffice, but that proved obstinate and unworkable; whereas, the reduced **13** and **14** reacted satisfactorily. The last were obtained by catalytic hydrogenation of the dipyrrolethene precursors (**11** and **12**) which were synthesized from the known monopyrroles (**7** and **8**, respectively) by McMurry coupling. Thus, as outlined in Scheme 2, the $\alpha\text{-CH}_3$ of **7** and **8** was oxidized to $\alpha\text{-CHO}$ (**9** and **10**) [26, 27], and **9** and **10** were each self-condensed using Ti^0 [23] in the McMurry coupling [16] procedure to afford dipyrrolethenes **11** and **12**. These tetra-esters were saponified to tetra-acids, but attempts to condense either of the latter with the designated (bromomethylene)pyrrolinone met with resistance, and no product like **3e** or **4e** could be isolated. Apparently decarboxylation of the $\alpha\text{-CO}_2\text{H}$ groups of saponified **11** and **12** did not occur. Attempts simply to decarboxylate the tetra-acids of **11** and **12** to provide the α -free 1,2-dipyrrolethenes were similarly unsuccessful, and we attributed the stability of the tetra-acids to the presence of the -CH=CH- group connecting the two pyrroles. Reducing the -CH=CH- to $\text{-CH}_2\text{-CH}_2\text{-}$ provided a way to overcome the problem of decarboxylation [16]. Thus, **11** and **12** were subjected to catalytic hydrogenation, the progress of which was monitored visually, for in solution the 1,2-bis(pyrrolyl)ethenes produce a blue fluorescence in the presence of Pd(C) , and when the mixture turns dark black, there is no observable fluorescence and reduction is therefore complete. Due to its poor solubility in most organic solvents, **11** had to be added in small portions during hydrogenation in order to prevent undissolved **11** from deactivating the catalyst. In contrast, **12** presented no solubility problems. The dipyrrolethanes from **11** and **12** were saponified to tetra-acids **13** and **14** in high yield. Coupling either of the latter with the 5-(bromomethylene)-3-pyrrolin-2-one proceeded smoothly, following in situ $\alpha\text{-CO}_2\text{H}$ decarboxylation, to provide the yellow-colored dimethyl esters (**1e** and **2e**), of **1** and **2**, respectively. The expectedly yellow-colored free acids (**1** and **2**) were easily obtained from their dimethyl esters by mild saponification.

Homoverdin synthesis aspects

For expected ease of handling and work-up, dehydrogenation was first attempted by reacting the dimethyl esters (**1e** and **2e**) of **1** and **2** with 2,3-dichloro-5,6-dicyano-1,4-quinone (DDQ). Thus, as in Scheme 2 treatment of **1e** in tetrahydrofuran (THF) for 2 h at room temperature with excess oxidizing agent (2 molar equivalents) resulted in but one main product in 42% isolated yield after easy purification by radial chromatography on silica gel. It was identified (vide infra) as the red-violet colored dehydro-*b*-homoverdin **5e**. In contrast, a

shorter reaction time (20 min) using the same stoichiometry afforded a violet-colored mixture of *b*-homoverdin **3e** and its dehydro analog **5e** in a 70:30 ratio. In order to maximize the yield of **3e** (and minimize that of **5e**), we found that one molar equivalent of DDQ in THF and a 60-min reaction time at room temperature afforded **3e** in 81% isolated yield. Dimethyl ester **2e** behaved quite similarly, yielding **4e6e**, or a mixture of **4e** and **6e**, depending analogously, on stoichiometry and reaction time. In separate experiments, as expected, treatment of **3e** (or **4e**) with DDQ produced **5e** (or **6e**). Unfortunately, saponification attempts on methyl esters **3e-6e** using NaOH or K₂CO₃ resulted in decomposition; so we turned to preparing **3-6** more directly.

Direct conversion of **1** and **2** to their corresponding *b*-homoverdins (**3** and **4**) was achieved by heating with DDQ, which was also expected to convert some of the first-formed **3** and **4** to their corresponding dehydro-*b*-homoverdins (**5** and **6**). Although mixtures might therefore have been expected, reaction of **1** with 2.5 molar equivalents of DDQ in (CH₃)₂SO at room temperature led to the immediate appearance of a blue color and after 30 min afforded only red-orange **3** (in 50% isolated yield). Similarly, **2** gave only red-orange **4**, in 47% isolated yield. Attempts to convert **1** or **2** to **5** or **6** by longer reaction times with DDQ, or by warming resulted only in pigment destruction and no apparent production of **5** or **6**.

As an alternative route to **5** and **6** and **2** were converted to *t*-butyldiphenylsilyl diesters with *t*-butyldiphenylsilyl chloride and oxidized with DDQ to give the corresponding *b*-homoverdin (**3** and **4**) and dehydro-*b*-homoverdin (**5**) diesters. The diester of **6** could not be obtained. Whereas, deprotection of the silyl esters using tetra-*n*-butylammonium fluoride in dry THF afforded **3** and **4**, only a trace of **5** was obtained.

Molecular structure

The constitutional structures of the (yellow) homorubin esters (**1e** and **2e**) follow from the method of synthesis and are in complete agreement with their ¹³C NMR spectra (Table 1). The chemical shifts of **1e** and **2e** correlate well with each other and with those from their mesobilirubin-XIII_a dimethyl ester analogs: **1e** and **2e** relative to mesobilirubin-XIII_a dimethyl ester itself (MBRe). Only small differences in chemical shifts are seen. Likewise, the ¹³C NMR chemical shifts of **1** and **2** correlate well with their structures and with those of the analogous mesobilirubin (Table 2).

The constitutional structures of the homoverdin and dehydro-homoverdin esters were also assigned on the basis of their ¹³C NMR data (Table 3). One finds the expected deshieldings for the ¹³C signals at C(10)/C(10a), C(8)/C(12), and C(9)/C(11), and the expected shieldings at C(2)/C(18) of **3e** and **4e** relative to **1** and **2**, due to the presence of the C(10)=C(10a) double bond. In **5e** and **6e**, the presence of the exocyclic double bonds at C(9)=C(10)/C(10a)=C(11), and the imino C=N bonds at C(6)/C(14) causes a striking deshielding of the C(9)/C(11) and C(6)/C(14) carbons in the dehydro-*b*-homoverdins (**5e6e**) relative to the *b*-homoverdins (**3e4e**). In **5e** and **6e**, the strongly deshielded carbon chemical shifts of C(6)/C(14) are characteristic of a C=N bond [28, 29], as are the deshielded chemical shifts for C(9)/C(11) [29, 30]. The extra conjugation of the former also perturbs the C(2)/C(18) and the C(7)/C(13) ¹³C NMR resonances, leading to similarly large deshieldings relative to the

b-homoverdins. Also noticeable are the greater deshieldings of the C(10)/C(10a) vinylic hydrogens of the dehydro-*b*-homoverdins relative to the *b*-homoverdins. Additional support for the assigned structures comes from exact-mass determinations of their molecular weights, e.g., for **3e** and **5e**. Fast atom bombardment high resolution mass spectrometry (FAB-HRMS) applied to homoverdins **3e** and **5e** of this work yielded the following high resolution molecular ion determinations: 626.3084 for **5e** (which is a good fit to the 626.3104 calculated for C₃₆H₄₂N₄O₆), and 628.3254 for **3e** (which is a good fit for the 628.3261 calculated for C₃₆H₄₄N₄O₆).

Our structure assignment of *b*-homoverdin differs from that of Chen et al. [19], who reinvestigated the reaction of the dipyrinone, kryptopyrromethenone, in CH₂Cl₂ with Br₂, a reaction previously conducted by Daroca et al. [31]. Though Fischer and Adler [32] had reported the conversion of xanthobilirubinic acid to mesobilirubin-XIII_a by reaction with Br₂ in acetic acid; interestingly, with a change of solvent from glacial acetic acid to CH₂Cl₂, Chen et al. discovered that reaction of methyl xanthobilirubinate with Br₂ in CH₂Cl₂ at room temperature led to the formation of a homoverdin, designated as a *b*-homoverdin and characterized as structure **3e**. Given the current availability of two clearly different homoverdin esters, **3e** and **5e**, both arising from oxidation of **1e** by DDQ, we took note of the fact that the NMR data (Table 3) of our **5e** corresponds better to the NMR data of the compound that Chen et al. called *b*-homoverdin dimethyl ester rather than to our **3e**. The strongly deshielded signal (~7.8 ppm) for the C(10)/C(10a) hydrogens also appears to correlate better to octamethyl-dehydro-*b*-homoverdin [20]; thus, we believe that the *b*-homoverdin assigned earlier [19] is more likely to be dehydro-*b*-homoverdin **5e**. Doubtless Chen et al. [19] were disadvantaged in not having both **3e** and **5e** available for comparison. In particular, one finds ¹³C NMR evidence for a C=N carbon-13 resonance from the pigment of Chen et al. more deshielded C(10)/C(10a) carbons and their hydrogens relative to our **3e** – but coincident with **5e**. It is puzzling that the soft ionization mass spectrometric molecular ion determinations (chemical ionization, CIMS, and fast atom bombardment high resolution, FABHRMS) by Chen et al. yielded 628.3265 (FAB-HRMS) for their homoverdin, corresponded to C₃₆H₄₄N₄O₆ (exact mass = 628.3260), hence the molecular weight of **3e** and not **5e**. This enigmatic and presumably misleading information is puzzlingly difficult to reconcile with a reassignment of their *b*-homoverdin assignment, unless the soft ionization method actually sampled traces of **3e** in a preponderantly **5e** sample – or unless the ionization method reduced some **5e** to **3e**.

Solution properties; chromatography

Homorubins **1** and **2** are yellow compounds, whose structures appear yellow in CHCl₃ with UV-Vis spectral characteristics very similar to mesobilirubins or dipyrinones (Table 1). They differ in color and in structure from their more conjugated *b*-homoverdins and their dimethyl esters (Table 5), which, e.g., in CHCl₃ are red-violet. Both homorubins **1** and **2** and *b*-homoverdins **3e** and **4e** also differ from their more unsaturated dehydro-*b*-homoverdin analogs **5e** and **6e**, which give blue-violet solutions in CHCl₃. Perhaps unexpectedly, the UV-Vis spectral characteristics of **3e** and **5e** differ little (Table 5).

The solubilities of the pigments varied considerably. While homorubin dimethyl esters (**1e** and **2e**) are soluble in a variety of nonpolar solvents, comparable to mesobilirubin dimethyl ester, the solubility of the free acids **1** and **2** closely resembles that of mesobilirubin: somewhat soluble in CHCl_3 and very soluble in $(\text{CH}_3)_2\text{SO}$, much less soluble in a range of organic solvents, and insoluble in H_2O . In contrast to the homorubin esters, the *b*-homoverdin dimethyl esters (**3e** and **4e**) are insoluble in CHCl_3 or CH_2Cl_2 but soluble in $\text{CH}_2\text{Cl}_2\text{-CH}_3\text{OH}$ and very soluble in $(\text{CH}_3)_2\text{SO}$. In further contrast, **5e** and **6e**, the dehydro-*b*-homoverdin dimethyl esters, are poorly soluble in $(\text{CH}_3)_2\text{SO}$ but soluble in CHCl_3 .

The *b*-homoverdin dimethyl ester solubility properties differ little from those of their free acids. Thus, the *b*-homoverdins are insoluble in non-polar organic solvents, though slightly soluble in the mixed $\text{CH}_2\text{Cl}_2\text{-CH}_3\text{OH}$ solvent, and quite soluble in $(\text{CH}_3)_2\text{SO}$ – in which they exhibit a deep red color similar to that of the dimethyl esters.

The pigment colors are not surprising. Consisting of two dipyrnone chromophores well-separated by their $-\text{CH}_2\text{-CH}_2-$ linker, **1** and **1e** and **2e** are expected to be yellow, as is observed. Though **3** and **3e** and **4e** also consist of two dipyrnones, one might expect them to be yellow-colored, were it not for the fact that they are linked by a $-\text{CH=CH}-$ unit, through which conjugation might be anticipated. Their red-orange color gives evidence to some level of electronic interaction of the dipyrnone chromophores through the ethene π -system. And in this case, the situation appears to be analogous to that observed when dipyrnones are linked by an ethyne ($-\text{C}\equiv\text{C}-$) unit, which also gives red-orange solutions, as was observed previously [33]. The dehydro-*b*-homoverdins [19, 20] exhibited the reddish color associated with the dipyrromethene chromophore [30, 34] and with α -benzylidene dipyrnones [35, 36].

Using chromatography as an indication of the relative polarity of homorubins **1** and **2**, and compared to mesobilirubin-XIII α , thin layer chromatography (TLC) revealed quite comparable R_f values, especially for **2** and mesobilirubin. Reversed phase performance liquid chromatography (HPLC) [10, 11] likewise similarly revealed very similar retention times for **2** and mesobilirubin. Homorubin **1**, while exhibiting the expected chromatographic behavior for a nonpolar rubin, appears to be slightly more polar than **2**; yet, all these data (Table 6) point to good intramolecular hydrogen bonding in **1** and **2**, as is well-known for mesobilirubin.

Homorubin conformational analysis and circular dichroism

Insight into the conformational structures of homorubins **1** and **2** may be gained from an inspection of their N-H proton NMR chemical shifts. Previously it was learned that in solvents which promote hydrogen bonding, such as CDCl_3 , dipyrnones are strongly attracted to engage in self association using hydrogen bonds [37, 38], except when a carboxylic acid group is available, for dipyrnones seem to be perfect hosts for the CO_2H group of acids [2, 8, 39–43]. When engaged in hydrogen bonding with a carboxylic acid group, the lactam N-H chemical shift tends to lie near 10.5 ppm, and the pyrrole N-H near 9 ppm in CDCl_3 . A good correlation was found from the N-H chemical shifts observed (Table

7) for **1** and **2**, which are consistent with intramolecular hydrogen bonding of the type seen in bilirubin (Fig. 1) and mesobilirubin in CDCl_3 .

The available evidence from diverse sources, NMR spectroscopy, solubility, and chromatographic properties is consistent with intramolecular hydrogen bonding between the polar carboxylic acid groups and dipyrinones of homorubins **1** and **2**, as in bilirubin and mesobilirubin, cf. Fig. 1B. In the homorubins, the stable (4Z,15Z) configuration of the dipyrinone units is maintained, consistent with nuclear Overhauser effects (NOEs) detected between the lactam and pyrrole NHs, and between C(5)H/C(15)H and the neighboring ethyls at C(8)/C(17). The three-dimensional shapes of the homorubins necessarily differ from that of bilirubin because they have an $-\text{CH}_2-\text{CH}_2-$ group rather than a $-\text{CH}_2-$ connecting the two dipyrinones, thereby imparting a third degree of rotational freedom about the center of the molecule. Consistent with the NOE study, and the N-H chemical shift data (Table 5) that support intramolecular hydrogen bonding, even with this increased level of molecular flexibility about C(10)/C(10a), the homorubins easily fold into and adopt conformations wherein their dipyrinones can come into hydrogen-bonding contact with the opposing alkanolic acids, as shown in Fig. 1F. The energy-minimized structures from Sybyl molecular dynamics computations [2] are shown, however, not to be planar. Like bilirubin, **1** and **2** fold into a three-dimensional intramolecularly hydrogen-bonded conformation. However, unlike bilirubin the shape is not like a ridge-tile. The planes containing the dipyrinones can adopt a more nearly parallel orientation, given two sp^3 -hybridized carbons connecting them. And with the extra degree of rotational freedom about the $-\text{CH}_2-\text{CH}_2-$ unit, the dipyrinones can rotate independently about each $-\text{CH}_2-$ group, and the ethylene group can rotate about its C(10)–C(10a) bond. Rotation about the latter tends to move the two dipyrinones into approximately transoid parallel planes (Fig. 2A), with the pyrrole rings stationed above and below each other. The minimum energy structures (Figs. 2B and C) shown in ball and stick representations (see Experimental) of homorubins **1** and **2** were computed to lie some $63\text{--}71\text{ kJ mol}^{-1}$ lower energy than the same folded conformation absent hydrogen bonds – an energy lowering comparable to that computed for bilirubin and mesobilirubin [2].

Although only small differences were detected between the UV-Vis spectra of **1** and **2**, and mesobilirubin-XIII α (Table 4), their CD spectra in CHCl_3 with added quinine differed substantially (Table 8). Under such conditions, mesobilirubin-XIII α gave an intense bisignate Cotton effect; whereas, any Cotton effects ($\epsilon > 0.1$) were hard to detect for **1** and **2**. In contrast, **1** in aq. buffered human serum albumin (HSA) [44–46] produced a very large bisignate CD, typical of exciton coupling [2, 44], with the same signed order and twice the intensity found for mesobilirubin-XIII α . In further contrast, the bisignate CD seen for **2** is only weak, of nearly an order of magnitude reduced in intensity relative to **1**. The CD (and UV-Vis) characteristics of bichromophore systems undergoing exciton coupling are dependent on the relative orientation of the induced electric dipole moments associated with the relevant electronic transition(s), in this case the $\sim 420\text{ nm}$ long wavelength transition. Since the intensity of the CD transitions depends both on orientation [2, 44] and enantiomeric excess of the pigment held in chiral conformations, the greatly reduced CD intensities of **2** on HSA probably reflect poor enantioselection by the binding protein or, less

likely, an unfavorable orientation of the dipyrinones (and the long wavelength electric transition dipoles) where the transition moments come close to being in-line or parallel.

b-Homoverdin conformational analysis

In both **3** and **4**, as well as in **3e** and **4e**, two configurational stereo-isomers are possible in *b*-homoverdins: either (*Z*) or (*E*) at the C(10)=C(10a) double bond (Fig. 3). We could not, however, determine the exact double bond stereochemistry experimentally. In their *b*-homoverdin studies, Chen et al. [19] tentatively assigned a (*Z*) configuration at C(10)=C(10a) based on the observation that the protons on the double bond were deshielded to $\delta \sim 7.8$ ppm relative to those ($\delta \sim 6.6$ ppm) of “a series of dipyrrolethenes of (*E*) configuration” [47]. Assuming that the $\delta \sim 6.6$ ppm indicates an (*E*)-configuration [48], one is tempted to assign (*E*) configurations to both **3e** and **4e**, based on the chemical shifts ($\delta \sim 6.8$ ppm) of their hydrogens at C(10)/C(10a). Given rotational degrees of freedom about the C(9)–C(10) and C(10a)–C(11) single bonds, one can imagine numerous conformations, of which a few (planar) are shown in Fig. 3.

In both diastereoisomers of **3** and **4**, given the possibility of rotation about the C(9)–C(10) and C(10a)–C(11) bonds, intramolecular hydrogen bonding appears to be possible, though we noted that the *b*-homoverdins are more polar (e.g., insoluble in CH₂Cl₂) than the corresponding homorubins (soluble in CH₂Cl₂). This may suggest less compact structures for **3** and **4** than **1** and **2** and support the (10*E*) configuration of the former pair. CPK molecular models of the *syn*-(10*E*)-*syn* reveal a flattened bowl shape and the possibility of intramolecular hydrogen bonding between each dipyrinone and an opposing propionic or butyric acid, although the acid carbonyls are somewhat buttressed against the C(10) and C(10a) hydrogens. From an inspection of models, intramolecular hydrogen bonding would seem less feasible in the *anti*-(10*E*)-*anti* and *anti*-(10*Z*)-*anti* conformations. The best conformation for intramolecular hydrogen bonding, with minimal non-bonding steric destabilizing interactions appears to be the *syn*-(10*Z*)-*syn* conformer, but only when the dipyrinones are rotated *synclinal*, with the C(8)–C(9)–C(10)=C(10a) and C(10)=C(10a)–C(11)–C(12) torsion angles approaching 90°. This is seen in the structures of Fig. 4.

Molecular mechanics calculations (Sybyl) predict that intramolecular hydrogen bonding between the dipyrinones and opposing propionic acids of **3** or the butyric acids of **4** (Fig. 4) stabilizes certain conformations of their (10*E*) and (10*Z*) isomers. The (10*Z*) isomers of **3** and **4** are predicted to be stabilized by ~ 81 and ~ 127 kJ mol^{–1}, respectively. In contrast, intramolecular hydrogen bonding is predicted to stabilize the (*E*) isomers of **3** and **4** by 57 kJ mol^{–1} and ~ 208 kJ mol^{–1}. From these data, one might think that for **3** intramolecularly hydrogen bonded (10*Z*) would be slightly more stable than intramolecularly hydrogen bonded (10*E*), and that for **4** (10*E*) would be much more stable than (10*Z*). As shown in Fig. 4, the (10*Z*) isomers fold into very different shapes from the (10*E*), where, as might be expected from an (*E*) C=C, the dipyrinones lie nearly in the same plane, giving the molecule an extended look. However, neither the (10*Z*) nor the (10*E*) isomer in the intramolecularly hydrogen-bonded conformations of Fig. 4 would seem to hint at their relative stabilities, nor do the torsion angles (Table 9). One might view the longer lactam N–H to carboxylic acid C=O hydrogen bond (b) of (10*E*)-**3** compared to (10*Z*)-**3** as indicating

less effective stabilization due to hydrogen bonding in the former. However, this assumes (reasonably) that an amide to CO₂H hydrogen bond is more stabilizing than a pyrrole to CO₂H, which is longer in (10Z)-**3** than in (10E)-**3**. A similar rationalization according less stabilization due to the longer N-H to acid C=O hydrogen bond of (10Z) vs. (10E) in **4** would suggest that the (10E) is more stable than the (10Z). It would appear that the longer butyric acid chain is more accommodating than propionic acid to intramolecular hydrogen bonding in the (10E) isomers. However, whether it is only the relative ability to engage in intramolecular hydrogen bonding as effectively as in mesobilirubin that serves to explain the differences in stability is unclear. In the conformations represented in Fig. 4, the acid chains all appear to adopt staggered conformations; therefore, one might conclude that the energies associated with intramolecular non-bonded steric compression also contribute to the relative differences in stability. Unfortunately, given the insolubility of **3** and **4** in CDCl₃ or CD₂Cl₂, we could not obtain their ¹H NMR spectra and employ the usual criteria of NH and CO₂H chemical shifts and CO₂H to NH NOEs to confirm intramolecular hydrogen bonding.

Dehydro-*b*-homoverdin conformation

Unlike the *b*-homoverdins, with a “rigid” (*Z*) or (*E*) C=C in the center of the molecule and two degrees of rotational freedom (about the C(9)–C(10) and C(10a)–C(11) single bonds), dehydro-*b*-homoverdins have but one rotatable bond in the center, the C(10)–C(10a) single bond. With two double bonds just off the center of the molecule vs. one in the center of *b*-homoverdins, three diastereomers are possible for the dehydro-*b*-homoverdins: (*Z,Z*), (*Z,E*), and (*E,E*), as illustrated in Fig. 5. As in biliverdin, mesobiliverdin, and related analogs [30], it can be assumed that the lactam NH to isopyrrole N is strong, with the hydrogen relatively unavailable for additional hydrogen bonds, e.g., to a carboxylic acid. And while many different conformations are possible for **5** and **6** due to rotation about the C(10)–C(10a) bond, we considered only those where non-bonding steric interactions are minimized and those that might be stabilized by residual, weak intramolecular hydrogen bonding between the carboxylic acids and opposing dipyrinones, as predicted by (Sybyl) molecular mechanics computations (Fig. 6) and observed in CPK molecular models. These included the more fully hydrogen-bonded *s-trans* and *s-cis* (9Z,10aZ) conformers (Figs. 5 and 6); however, the preference for such conformations could not be confirmed experimentally, and the various bond angles and hydrogen bond distances (Table 10) found in the minimum energy structures of Fig. 6 do not offer clarification.

Concluding Comments

In connection with our interest in centrally expanded [11, 16, 33, 35, 50–52] and contracted [53] analogs of the synthetic model (mesobilirubin-XIII α) for the natural pigment of human bile and jaundice [1], we prepared homorubin **1** and its analog **2**, with butyric acid groups replacing propionic acids. Yellow **1** and **2** preferentially adopt folded, intramolecularly hydrogen-bonded conformations and exhibit a lipophilicity comparable to that of mesobilirubin-XIII α . Like the last, hepatobiliary elimination of homorubin **1** in Sprague-Dawley rats [10, 11, 54] succeeds by formation of mono- and diglucuronides, as well (surprisingly) as intact [16]. The reddish *b*-homoverdins (**3** and **4**) corresponding to **1** and **2**,

which can exist as (10*Z*) or (10*E*) diastereomers, prefer the latter and are stabilized by intramolecular hydrogen bonds.

Experimental

All nuclear magnetic (NMR) spectra were obtained on a Varian unity plus at 11.75 T magnetic strength operating at 500 MHz (^1H) and 125 MHz (^{13}C), QN 400 MHz (^1H) and 100 MHz (^{13}C), and Varian GE at 7.06 T magnetic strength operating at 300 MHz (^1H) and 75 MHz (^{13}C), respectively, in deuteriochloroform unless otherwise indicated. Chemical shifts were reported in ppm referenced to the residual chloroform proton signal at 7.26 ppm and ^{13}C at 77.23 ppm unless otherwise noted. A combination of heteronuclear multiple bond correlation (HMBC) spectra, heteronuclear single bond correlation (HSQC) spectra, two-dimensional correlation spectroscopy (COSY), and $^1\text{H}\{^1\text{H}\}$ nuclear Overhauser effect (NOE) data were used to assign ^1H and ^{13}C NMR spectra. Melting points were taken on a Mel-Temp capillary apparatus. Analytical samples were dried under vacuum in a drying pistol (Abderhalden) at refluxing ethanol or toluene temperature using P_2O_5 as desiccant. Combustion analyses were performed by Desert Analytics, Tucson, AZ and gave results within $\pm 0.4\%$ of theoretical values. For a few compounds, FAB-HRMS mass determinations of the molecular ion were obtained from the Nebraska Center for Mass Spectrometry, Lincoln, Nebraska. UV-Vis spectra were recorded on a Perkin-Elmer Lambda-12 spectrometer. Circular dichroism spectra were measured on a Jasco J-600 spectrometer. Vapor pressure osmometry (VPO) measurements were performed on an OSMOMAT 070-SA instrument (Gonotech GmbH, Germany) in HPLC grade CHCl_3 (Fisher) at 45 °C with benzil used for calibration in CHCl_3 and methanol and (+)-10-camphorsulfonic acid for calibration in water. For final purification, radial chromatography was carried out on Merck silica gel PF₂₅₄ with gypsum binder, preparative layer grade, using a Chromatotron (Harrison Research, Palo Alto, CA). Analytical thin layer chromatography was carried out on J.T. Baker silica gel IB-F plates (125 μ layers). Flash chromatography was carried out using Woelm silica gel F, thin layer chromatography grade. HPLC analyses were carried out on a Perkin-Elmer Series four high performance liquid chromatograph with an LC-95 UV-Vis spectrophotometric detector (set at 420 nm or 640 nm) equipped with a Beckman-Altex ultrasphere-IP 5 μm C-18 ODS column (25 $\times$ 0.46 cm). The flow rate was 1.0 cm^3/min , and the elution solvent was 0.1 M di-*n*-octylamine acetate in 5% aq. methanol (pH 7.7, 35 °C). All reagents and solvents used in the syntheses were obtained from Fisher-Acros, Aldrich, and Alfa Aesar. Deuterated chloroform, dichloromethane, dimethylsulfoxide, and methanol were from Cambridge Isotope Laboratories. Molecular dynamics computations were performed on a SGI Octane workstation using versions 6.9 and 7.1 of SYBYL (Tripos Assoc., St. Louis, MO) force field with Gasteiger-Hückel charges. Ball and stick drawings were created from atomic coordinates of the molecular dynamics structure using Müller and Falk's "Ball and Stick" program (Cherwell Scientific, Oxford, U.K.) for the Macintosh (http://www.orc.uru-Linz.ac.at/mueller/ball_and_stick.shtml). All solvents were reagent grade, from Fisher-Acros.

Some synthetic precursors were available from previous work [49]: ethyl 5-(ethoxycarbonyl)-2,4-dimethyl-1*H*-pyrrole-3-propanoate (**7**) and the corresponding 3-butanoate (**8**).

(4Z,15Z)-2,2'-(1,2-Ethanediy)bis[5-[(3-ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-¹H-pyrrole-3-propanoic acid] (1C₃₄H₄₂N₄O₆)

To a solution of 0.08 g homorubin dimethyl ester **1e** (0.13 mmol) in 10 cm³ THF and 3 cm³ CH₃OH, 2.5 cm³ of a 1 M aq. NaOH solution was added, and the solution was heated at reflux for 3 h under an inert atmosphere. The reaction was quenched by pouring the solution into an ice-water bath followed by acidification with aq. NaHSO₄ to pH 4. The acidified solution was extracted with CH₂Cl₂ (2 × 100 cm³), and the CH₂Cl₂ solution was dried over anhydrous Na₂SO₄, and evaporated in vacuo (rotovap). The solid residue was triturated with 3 cm³ CH₃OH, and the resulting yellow solid was removed by filtration to afford pure **1**.

Yield: 60 mg (85%); m.p.: 220–221 °C (dec); ¹H NMR ((CD₃)₂SO): δ = 1.10 (6H, t, *J* = 7.3 Hz), 1.86 (6H, s), 2.12 (6H, s), 2.45 (4H, q, *J* = 7.3 Hz), 2.75 (4H, t, *J* = 7.3 Hz), 2.86 (4H, t, *J* = 7.3 Hz), 3.34 (4H, s), 6.00 (2H, s), 8.59 (2H, brs), 10.18 (2H, brs), 13.94 (2H, brs) ppm; ¹³C NMR data in Table 2; UV-Vis data in Table 4; CD data in Table 8.

(4Z,15Z)-2,2'-(1,2-Ethanediy)bis[5-[(3-ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-¹H-pyrrole-3-propanoic acid] dimethyl ester (1eC₃₆H₄₆N₄O₆)

2,2'-(1,2-Ethanediy)bis[5-(ethoxycarbonyl)-4-methyl-1*H*-pyrrole-3-propanoic acid] (**13217** mg, 0.49 mmol) was dissolved in 30 cm³ 20 CH₃OH in a 100 cm³ 21 round bottom flask. To this solution were added 209 mg 5-(bromomethylene)-3-pyrrolin-2-one (**150.968** mmol) and a drop of aq. HBr. The resulting mixture was stirred and heated at reflux for 15 h during which time a green solid developed in the reaction mixture. The green solid was isolated by filtration, dissolved in CH₂Cl₂, and further purified by radial chromatography using 98:2 CH₂Cl₂:CH₃OH (by vol) as eluent to afford pure **1e**. Yield: 135 mg (41%); m.p.: 235 °C; ¹H NMR (300 MHz): δ = 1.02 (6H, t, *J* = 7.5 Hz), 1.18 (6H, s), 2.10 (4H, s), 2.32 (4H, q, *J* = 7.5 Hz), 2.53 (4H, t, *J* = 7.5 Hz), 2.82 (4H, t, *J* = 7.5 Hz), 3.12 (4H, s), 3.72 (6H, s), 5.85 (2H, s), 10.27 (2H, brs), 11.0 (2H, brs) ppm; ¹³C NMR data in Table 1.

(4Z,15Z)-2,2'-(1,2-Ethanediy)bis[5-[(3-ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-¹H-pyrrole-3-butanoic acid] (2C₃₆H₄₆N₄O₆)

To a solution of 0.15 g homorubin dimethyl ester **2e** (0.23 mmol) in 10 cm³ THF and 3 cm³ CH₃OH, 2.5 cm³ 1 M aq. NaOH solution was added, and the solution was treated and worked up as for **1e**. The precipitate formed was collected by filtration under aspirator pressure and was triturated with CH₂Cl₂ then filtered to give pure **2**. Yield: 110 mg (83%); m.p.: 285 °C (dec); ¹H NMR ((CD₃)₂SO): δ = 1.09 (6H, t, *J* = 7.0 Hz), 1.40 (4H, m), 1.75 (6H, s), 2.10 (6H, s), 2.14 (4H, t, *J* = 7.3 Hz), 2.30 (4H, m), 2.44 (4H, s), 2,2'-(1,2-Ethanediy)bis[5-(ethoxycarbonyl)-4-methyl-1*H*-pyrrole-3-propanoic acid] (**13217** mg, 0.49 mmol) was dissolved in 30 cm³ CH₃OH in a 100 cm³ round bottom flask. To this solution were added 209 mg 5-q, *J* = 7.0 Hz), 2.48 (4H, t, *J* = 7.3 Hz), 2.79 (4H, s), 5.93 (2H, s), 9.84 (2H, brs), 10.12 (2H, brs) ppm; ¹³C NMR data in Table 2; UV-Vis data in Table 4; CD data in Table 8.

(4Z,15Z)-2,2'-(1,2-Ethanediy)bis[5-[(3-ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-¹H-pyrrole-3-butanoic acid] dimethyl ester (2eC₃₈H₅₀N₄O₆)

2,2'-(1,2-Ethanediy)bis[5-(ethoxycarbonyl)-4-methyl-1*H*-pyrrole-3-butanoic acid] (**14686** mg, 1.53 mmol) was dissolved in 30 cm³ CH₃OH in a 100 cm³ round bottom flask to which 662 mg 5-(bromomethylene)-3-pyrrolin-2-one (**153.07** mmol) and 3–4 drops aq. HBr were added. The resulting mixture was stirred and heated at reflux for 20 h, during which a green solid developed in the reaction mixture. The solid was isolated by filtration and characterized as the desired product **2e**. Yield: 250 mg (25%); m.p.: 239–240 °C; ¹H NMR: δ = 1.09 (6H, t, *J* = 7.0 Hz), 1.20 (6H, s), 1.85 (4H, quint, *J* = 7.0 Hz), 2.10 (6H, s), 2.32 (4H, q, *J* = 7.2 Hz), 2.41 (4H, t, *J* = 7.2 Hz), 2.52 (3H, t, *J* = 7.2 Hz), 3.12 (4H, s), 3.70 (6H, s), 5.86 (2H, s), 10.27 (2H, brs), 11.03 (2H, brs) ppm; ¹³C NMR data in Table 1.

(4Z,15Z)-2,2'-(1,2-Ethenediy)bis[5-[(3-ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-¹H-pyrrole-3-propanoic acid] dimethyl ester (3eC₃₆H₄₄N₄O₆)

Homorubin dimethyl ester **1e** (40 mg, 0.063 mmol) was dissolved in 30 cm³ THF under an N₂ atmosphere. Then 14 mg DDQ (0.061 mmol) in 5 cm³ THF was added, and the mixture was stirred for 60 min. The reaction mixture was then poured into 100 cm³ ice-cold water containing 100 mg ascorbic acid. The resulting mixture was extracted with CH₂Cl₂ (3 × 75 cm³). The combined CH₂Cl₂ extractions were washed with saturated aq. NaHCO₃, dried over sodium sulfate, and evaporated to give crude **3e**. The crude product was purified using radial chromatography using 99:1 CH₂Cl₂:CH₃OH (by vol). Yield: 33 mg (81%); m.p.: 250 °C (dec); IR (KBr): $\bar{\nu}$ = 3424, 2942, 2355, 1734, 1654, 1625, 1460, 1260, 1160 cm⁻¹; ¹H NMR: δ = 1.10 (6H, t, *J* = 7.5 Hz), 1.95 (6H, s), 2.05 (6H, s), 2.50 (4H, q, *J* = 7.2 Hz), 2.50 (4H, t, *J* = 7.5 Hz), 2.80 (4H, t, *J* = 7.5 Hz), 3.60 (6H, s), 5.90 (2H, s), 6.90 (2H, s), 10.20 (2H, brs), 10.30 (2H, brs) ppm; ¹³C NMR data in Table 3; UV-Vis data in Table 5; FAB-HRMS: exact mass calculated for C₃₆H₄₄N₄O₆ 628.3261, found 628.3254.

(4Z,15Z)-2,2'-(1,2-Ethenediy)bis[5-[(3-ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-¹H-pyrrole-3-propionic acid] (3C₃₄H₄₀N₄O₆)

In a 25 cm³ round bottom flask 20 mg **1** (0.033 mmol) was dissolved in 10 cm³ distilled dimethyl sulfoxide. DDQ (17 mg, 0.083 mmol) in 2 cm³ dimethyl sulfoxide was added at once, and the solution was allowed to stir for 30 min (upon addition of the DDQ the solution immediately turned a blue color). The solution was poured into 50 cm³ ice water containing 100 mg ascorbic acid, and a precipitate formed. The precipitate was separated and washed by centrifugation and isolated by filtration. The solid was dried (high vacuum), dissolved in CH₂Cl₂:CH₃OH (90:10 by vol), and eluted through a column of silica using CH₂Cl₂:CH₃OH (93:7 by vol). A deep red compound was collected. The solvent was removed giving pure **3**. Yield: 10 mg (50%); m.p.: 276 °C; IR (KBr): $\bar{\nu}$ = 3444, 2970, 1669, 1636, 1386, 1265, 1168, 981, 758, 669 cm⁻¹; ¹H NMR ((CD₃)₂SO): δ = 1.07 (6H, t, *J* = 7.3 Hz), 1.77 (6H, s), 2.04 (6H, s), 2.33 (4H, t, *J* = 7.3 Hz), 2.51 (4H, q, *J* = 7.3 Hz), 2.76 (3H, t, *J* = 7.3 Hz), 5.94 (2H, s), 6.88 (2H, s), 10.17 (2N-H, bs), 10.28 (2N-H, bs), 12.20 (2COOH, vbs) ppm; ¹³C NMR ((CD₃)₂SO): δ = 8.61, 9.68, 15.33, 17.63, 20.00, 35.63, 97.23, 113.41, 123.57, 124.04, 124.17, 125.79, 129.86, 132.54, 147.55, 172.56, 174.40 ppm; UV-Vis data in Table 5.

(4Z,15Z)-2,2'-(1,2-Ethenediyl)bis[5-[(3-ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-1H-pyrrole-3-butanoic acid] dimethyl ester (4eC₃₈H₄₈N₄O₆)

Homorubin dimethyl ester **2e** (40 mg, 0.061 mmol) was treated as in the synthesis of **3e** above to give crude **4e**. The crude product was purified using radial chromatography using CH₂Cl₂:CH₃OH (99:1 by vol). Yield: 28 mg (72%); m.p.: 264 °C; ¹H NMR: δ = 1.10 (6H, t, *J* = 7.2 Hz), 1.70 (4H, quint, *J* = 7.5 Hz), 1.90 (6H, s), 2.05 (6H, s), 2.30 (4H, t, *J* = 7.5 Hz), 2.50 (4H, q), 2.60 (4H, t, *J* = 7.5 Hz), 3.55 (6H, s), 5.95 (2H, s), 6.90 (2H, s), 10.20 (2H, brs), 10.30 (2H, brs) ppm; ¹³C NMR in Table 3; UV-Vis data in Table 5; FAB-HRMS: calcd for C₃₈H₄₈N₄O₆ [M]⁺ 656.3574, found 656.3589.

4Z,15Z)-2,2'-(1,2-Ethenediyl)bis[5-[(3-ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-1H-pyrrole-3-butanoic acid] (4C₃₆H₄₆N₄O₆)

To a solution of 20 mg homorubin acid **2** (0.03 mmol) in 10 cm³ dry CH₃)₂SO 17 mg DDQ (0.083 mmol) was added at once, and the solution was allowed to stir for 15 min. The reaction mixture was then poured into ice-water and stirred in an ice bath. The resulting solid was then removed by suction filtration, dissolved in 10 cm³ CH₂Cl₂:CH₃OH (60:40 by vol), and purified by flash column chromatography on silica gel using CH₂Cl₂:CH₃OH (50:50 by vol) as eluent. The pure fractions were evaporated in vacuo to obtain pure **4**. Yield: 10 mg (47%); m.p.: 273 °C (dec); ¹H NMR ((CD₃)₂SO): δ = 1.10 (6H, t, *J* = 7.3 Hz), 1.75 (4H, m), 1.80 (6H, s), 2.07 (6H, s), 2.36 (4H, t, *J* = 7.0 Hz), 2.51 (4H, q, *J* = 7.3 Hz), 2.79 (4H, t, *J* = 7.0 Hz), 5.96 (2H, s), 6.90 (2H, s), 10.16 (2H, s), 10.29 (2H, s), 12.04 (2H, brs) ppm; UV-Vis data in Table 5.

(4Z,15Z)-9,9'-(1,2-Ethanedilylidene)bis[3-ethyl-1,9-dihydro-2,7-dimethyl-1-oxodipyrin-8-propionic acid methyl ester] (5eC₃₆H₄₂N₄O₆)

In a 50 cm³ round-bottom flask equipped with a magnetic stirrer was dissolved 40 mg homorubin dimethyl ester **1e** (0.063 mmol) in 30 cm³ THF. To this solution was added 32 mg DDQ (0.130 mmol). The mixture was stirred for 20 min, then quenched with 75 cm³ water containing 100 mg ascorbic acid, and extracted with 50 cm³ CH₂Cl₂. The CH₂Cl₂ extract was washed with 20 cm³ aq. 10% NaHCO₃, water (3 × 20 cm³), and dried over anhydrous Na₂SO₄. The CH₂Cl₂ was removed (rotovap), and the remaining solid was purified using radial chromatography (CH₂Cl₂:CH₃OH, 97:3 by vol), resulting in **5e** as a violet solid. Yield: 30 mg (76%); m.p.: 260 °C (dec); IR (KBr): $\bar{\nu}$ = 3436, 2954, 2919, 2355, 1701, 1648, 1625, 1601 cm⁻¹; ¹H NMR: δ = 1.20 (6H, t, *J* = 7.3 Hz), 1.95 (6H, s), 2.10 (6H, s), 2.53 (4H, q, *J* = 7.3 Hz), 2.61 (4H, t, *J* = 7.2 Hz), 2.90 (4H, t, *J* = 7.2 Hz), 3.67 (6H, s), 5.88 (2H, s), 7.75 (2H, s), 10.5 (2N-H, bs) ppm; ¹³C NMR in Table 3; UV-Vis data in Table 5; FAB-HRMS: exact mass calculated for C₃₆H₄₄N₄O₆ 626.3104, found 626.3084.

In a separate experiment, 40 mg homorubin dimethyl ester **1e** (0.063 mmol) was dissolved in 30 cm³ THF under an N₂ atmosphere. To it was added 28 mg DDQ (0.122 mmol) in 5 cm³ THF, and the reaction mixture was stirred for 2 h at room temperature. Then it was poured into 100 cm³ ice-cold water containing 100 mg ascorbic acid and extracted with CH₂Cl₂ (3 × 75 cm³). After the combined organic extracts were washed with sat. aq. NaHCO₃, the product was dried over anhydrous Na₂SO₄. The solvent was evaporated (rotovap) to give a violet-colored mixture of **3e** and **5e**, which was separated by radial

chromatography using CH₂Cl₂:CH₃OH (99:1 by vol) as eluent. The doubly oxidized product (**5e**) was less polar and moved faster in the chromatography as a violet band; whereas, the more polar singly oxidized product (**3e**) followed as a red-violet band. Yield of **5e**: 17 mg (42%); m.p.: 260 °C.

(4Z,15Z)-9,9'-(1,2-Ethanediyldiene)bis[3-ethyl-1,9-dihydro-2,7-dimethyl-1-oxodipyrin-8-butanoic acid methyl ester] (6eC₃₈H₄₆N₄O₆)

Homorubin dimethyl ester **2e** (40 mg, 0.061 mmol) was oxidized as in the conversion of **1e** to **5e** to give crude **6e**, which was purified by radial chromatography using CH₂Cl₂:CH₃OH (99:1 by vol). Yield: 13 mg (28%); m.p.: 271 °C; ¹H NMR: δ = 1.10 (6H, t, *J* = 7.2 Hz), 1.80 (4H, quint), 1.99 (6H, s), 2.10 (6H, s), 2.40 (4H, t, *J* = 7.2 Hz), 2.50 (4H, q, *J* = 7.2 Hz), 2.70 (4H, t, *J* = 7.2 Hz), 3.60 (6H, s), 5.80 (2H, s), 7.80 (2H, s), 10.50 (2H, brs) ppm; ¹³C NMR in Table 3; UV-Vis data in Table 5.

Ethyl 5-(ethoxycarbonyl)-2-formyl-4-methyl-¹H-pyrrole-3-propanoate (9C₁₄H₁₉NO)

Ethyl 2,4-dimethyl-5-(ethoxycarbonyl)-1*H*-pyrrole-3-propanoate (726.7 g, 0.10 mol), 15 cm³ THF, 150 cm³ glacial acetic acid, and 100 cm³ H₂O were added to a 1000 cm³ round bottom flask and stirred magnetically to dissolve the pyrrole. The solution was cooled to –5 °C using an ice-salt bath, and 219.3 g ceric ammonium nitrate (CAN, 0.40 mol) was added in portions. After the final addition, the reaction mixture was allowed to stir for 2 h. Then the reaction mixture was added to a 2000 cm³ separatory funnel containing 1000 cm³ water and extracted with 300 cm³ CH₂Cl₂. The organic extract was washed with 10% aq. NaHCO₃ (4 × 100 cm³) to remove excess acetic acid, separated, and dried over anhydrous Na₂SO₄. The solvent was removed in vacuo to give a crude product, which was purified by column chromatography on silica gel using CH₂Cl₂:CH₃OH (99:1 by vol) to give pure **9**. Yield: 24.7 g (88%); m.p.: 60–61 °C (Ref. [26, 42] 61–62 °C); ¹H NMR (300 MHz): δ = 1.25 (3H, t, *J* = 7.1 Hz), 1.38 (3H, t, *J* = 7.1 Hz), 2.30 (3H, s), 2.55 (2H, t, *J* = 7.1 Hz), 3.06 (2H, t, *J* = 7.1 Hz), 4.10 (2H, q, *J* = 7.1 Hz), 4.35 (2H, q, *J* = 7.1 Hz), 9.46 (¹H, brs), 9.81 (¹H, s) ppm; ¹³C NMR (75 MHz): δ = 9.8, 14.1, 14.3, 18.8, 35.3, 60.6, 60.9, 124.5, 126.6, 129.9, 132.1, 160.8, 172.1, 179.5 ppm.

Ethyl 5-(ethoxycarbonyl)-2-formyl-4-methyl-¹H-pyrrole-3-butanoate (10C₁₅H₂₁NO₅)

Ethyl 5-(ethoxycarbonyl)-2,4-dimethyl-1*H*-pyrrole-3-butanoate (828.1 g, 0.10 mol) was dissolved in 250 cm³ acetic acid in a 2000 cm³ round bottom flask. To it 150 cm³ THF and 200 cm³ H₂O were added, and the solution was cooled to –5 °C using an ice-salt bath. Then, 219.3 g CAN (0.40 mol) was added in portions. After the addition was complete, the reaction mixture was stirred for 3 h at 0 °C. Work-up and purification were accomplished following the procedure for the synthesis of **9**. Yield: 24.1 g (82%); m.p.: 48–49 °C; ¹H NMR (300 MHz): δ = 1.25 (3H, t, *J* = 7.2 Hz), 1.30 (3H, t, *J* = 7.2 Hz), 1.90 (2H, quint, *J* = 7.2 Hz), 2.23 (3H, s), 2.36 (2H, t, *J* = 7.2 Hz), 2.78 (2H, t, *J* = 7.2 Hz), 4.13 (2H, q, *J* = 7.2 Hz), 4.37 (2H, q, *J* = 7.2 Hz), 9.45 (¹H, brs), 9.73 (¹H, s) ppm; ¹³C NMR (75 MHz): δ = 9.7, 14.2, 14.3, 22.6, 26.4, 33.3, 60.4, 60.9, 124.6, 126.6, 130.0, 133.5, 160.9, 173.0, 179.2 ppm.

**2,2'-(1,2-Ethenediyl)bis[ethyl 5-(ethoxycarbonyl)-4-methyl-¹H-pyrrole-3-propanoate]
(11C₂₈H₃₈N₂O₈)**

In a three-neck 500 cm³ round bottom flask equipped with magnetic stirrer, condenser, and N₂ inlet, 200 cm³ THF (dry) and a stir bar were added, and the solvent was cooled to 0 °C using an ice-salt bath. Then 2 cm³ TiCl₄ (17 mmol) was added dropwise using a syringe, and a yellow precipitate formed. Stirring was continued for an additional 10 min, then 2.2 g Zn (34 mmol) was added in portions and stirred for 10 min. The resulting mixture was heated at reflux for 2 h when the color of the mixture changed from yellow to green to black. After refluxing, 1.0 g pyrrole aldehyde **9** (3.4 mmol) in 20 cm³ dry THF was added, and refluxing was continued for 2 h. The reaction mixture was cooled to 5 °C in an ice bath and quenched by the addition of 200 cm³ 50% NH₄OH with stirring. The reaction mixture was added to a separatory funnel and extracted into CH₂Cl₂. The organic layer was filtered to remove darkish TiO₂, and the yellow organic layer was washed with water then dried over anhydrous Na₂SO₄. The solvent was evaporated in vacuo to give an orange-yellow oil, which was crystallized from ethyl acetate and hexane to give pure **11**. Yield: 540 mg (57%); m.p.: 161–162 °C; ¹H NMR (300 MHz): δ = 1.30 (6H, t, *J* = 7.2 Hz), 1.55 (6H, t, *J* = 7.2 Hz), 2.30 (6H, s), 2.50 (4H, t, *J* = 7.5 Hz), 2.90 (4H, t, *J* = 7.2 Hz), 4.10 (4H, q, *J* = 7.2 Hz), 4.30 (4H, q, *J* = 7.2 Hz), 6.75 (2H, s), 9.0 (2H, brs) ppm; ¹³C NMR (75 MHz): δ = 10.3, 14.2, 14.5, 19.4, 35.4, 60.1, 60.5, 114.1, 119.8, 123.8, 127.3, 130.4, 161.5, 172.9 ppm.

**2,2'-(1,2-Ethenediyl)bis[ethyl-5-(ethoxycarbonyl)-4-methyl-¹H-pyrrole-3-butanoate]
(12C₃₀H₄₂N₂O₈)**

The synthesis of **12** was accomplished using pyrrole aldehyde **10** following the procedure for the synthesis of **11**. Yield: 0.480 g (61%); m.p.: 84–85 °C; ¹H NMR (CDCl₃, 300 MHz): δ = 1.26 (6H, t, *J* = 7.2 Hz), 1.37 (6H, t, *J* = 7.2 Hz), 1.8 (2H, quint, *J* = 7.5 Hz), 2.26 (6H, s), 2.36 (4H, t, *J* = 7.2 Hz), 2.55 (4H, t, *J* = 7.2 Hz), 4.20 (4H, q, *J* = 7.2 Hz), 4.35 (4H, q, *J* = 7.2 Hz), 6.82 (2H, s), 9.17 (2H, brs) ppm; ¹³C NMR (CDCl₃, 125 MHz): δ = 10.3, 14.3, 14.6, 23.1, 26.1, 33.3, 59.9, 60.4, 114.1, 120.1, 124.6, 127.2, 131.0, 162.2, 173.8 ppm.

**2,2'-(1,2-Ethenediyl)bis[5-(ethoxycarbonyl)-4-methyl-¹H-pyrrole-3-propanoic acid]
(13C₂₀H₂₈N₂O₈)**

Dipyrrolylene **11** (500 mg, 0.90 mmol) was dissolved in 25 cm³ ethyl acetate, to which 50 mg of 5% Pd/C was added, and the mixture was stirred under a hydrogen atmosphere for 12–36 h (until the fluorescence of the solution had disappeared). The reaction mixture was passed through a celite layer to remove the catalyst, which was washed with ethyl acetate. The solvent was removed in vacuo to afford a white solid, which was recrystallized from ethyl acetate and hexane to obtain the pure hydrogenated tetra-ester **13**. Yield: 498 mg (99%); m.p.: 128–130 °C; ¹H NMR (CDCl₃, 300 MHz): δ = 1.30 (6H, t, *J* = 7.2 Hz), 1.55 (6H, t, *J* = 7.2 Hz), 2.30 (6H, s), 2.50 (4H, t, *J* = 7.5 Hz), 2.75 (4H, t, *J* = 7.2 Hz), 2.90 (4H, s), 4.1 (4H, q, *J* = 7.2 Hz), 4.30 (4H, q, *J* = 7.2 Hz), 8.75 (2H, brs) ppm; ¹³C NMR (CDCl₃, 75 MHz): δ = 10.3, 14.0, 14.4, 19.1, 26.2, 35.0, 59.4, 59.5, 60.2, 60.2, 117.8, 120.2, 126.6, 132.3, 161.2, 173.0 ppm.

The hydrogenated tetra-ester dipyrrole (500 mg, 0.732 mmol) was dissolved in 20 cm³ ethanol and to it was added 25 cm³ of 1 M aq. NaOH. The reaction mixture was stirred and

heated at reflux for 3 h (reaction completion was confirmed by TLC on silica gel). The ethanol was removed in vacuo, and to the residue was added 10 cm³ water. After cooling in an ice-salt bath to 0 °C the solution was acidified by adding 50% HNO₃ dropwise until a white precipitate formed. Then 1 cm³ more HNO₃ was added, and the mixture was stirred in the cold for 30 min. The resulting brown solid tetra-acid **13** was collected by filtration. Yield: 382 mg (99%); m.p.: 150–152 °C (dec); ¹H NMR (CDCl₃, 300 MHz): δ = 2.00 (3H, s), 2.25 (2H, t, *J* = 7.5 Hz), 2.50 (2H, t, *J* = 7.5 Hz), 2.60 (2H, s), 10.9 (¹H, s), 11.9 (¹H, brs) ppm; ¹³C NMR (CDCl₃, 75 MHz): δ = 10.6, 19.4, 26.4, 35.6, 117.5, 119.7, 125.4, 133.8, 162.7, 174.3 ppm.

**2,2'-(1,3-Ethanediy)bis[5-(ethoxycarbonyl)-4-methyl-¹H-pyrrole-3-butanoic acid]
(14C₂₂H₃₂N₂O₈)**

Dipyrrolethene **12** (250 mg, 0.448 mmol) was converted into **14** following the procedure for the synthesis of **13**. Dipyrrolethane tetra-ester: yield: 245 mg (99%); m.p.: 103–104 °C; ¹H NMR (CDCl₃, 300 MHz): δ = 1.32 (6H, t, *J* = 7.2 Hz), 1.42 (6H, t, *J* = 7.2 Hz), 1.82 (4H, q, *J* = 7.2 Hz), 2.20 (6H, s), 2.32 (3H, t, *J* = 7.2 Hz), 2.45 (4H, t, *J* = 7.2 Hz), 2.90 (4H, s), 4.25 (4H, q, *J* = 7.2 Hz), 4.28 (4H, q, *J* = 7.2 Hz), 9.15 (2H, brs) ppm; ¹³C NMR (CDCl₃, 125 MHz): δ = 10.5, 14.2, 14.6, 23.3, 26.1, 26.7, 33.4, 59.5, 60.5, 117.6, 121.2, 126.8, 133.2, 161.9, 173.9 ppm.

Dipyrrolethane tetra-acid: yield: 192 mg (97%); m.p.: 152 °C (dec); ¹H NMR (CDCl₃, 500 MHz): δ = 1.48 (4H, q, *J* = 7.0 Hz), 2.06 (6H, s), 2.12 (4H, t, *J* = 7.0 Hz), 2.27 (4H, t, *J* = 7.0 Hz), 2.54 (4H, s), 10.87 (2H, brs), 11.88 (4H, brs) ppm; ¹³C NMR (CDCl₃, 125 MHz): δ = 10.8, 23.1, 26.5, 26.5, 33.7, 117.5, 120.6, 125.6, 133.8, 162.8, 174.8 ppm.

Acknowledgments

We thank the late Prof. A. F. McDonagh (Univ. California San Francisco) for the metabolism studies and the National Institutes of Health (R01 HD 17779) for partial support.

References

1. Chowdury, JF.; Wolkoff, AW.; Chowdury, NR.; Arias, IM. Hereditary jaundice and disorders of bilirubin metabolism. chap 125. In: Scriver, CR.; Sly, WS.; Childs, B.; Beaudet, AL.; Valle, D.; Kinzler, KW.; Vogelstein, B., editors. *The Metabolic and Molecular Bases of Inherited Disease*. 8th edn. New York: McGraw-Hill, Inc; 2001.
2. Person RV, Peterson BR, Lightner DA. *J Am Chem Soc*. 1994; 116:42.
3. Bonnett R, Davies JE, Hursthouse MB, Sheldrick GM. *Proc Royal Soc London*. 1978; B202:249.
4. LeBas G, Allegret A, Mauguén Y, DeRango C, Bailly M. *Acta Cryst*. 1980; B36:3007.
5. Mugnoli A, Manitto P, Monti D. *Acta Cryst*. 1983; C39:1287.
6. Kaplan D, Navon G. *Isr J Chem*. 1983; 23:177.
7. Nogales D, Lightner DA. *J Biol Chem*. 1995; 270:73. [PubMed: 7814422]
8. Dörner T, Knipp B, Lightner DA. *Tetrahedron*. 1997; 5:2697.
9. Boiadjev SE, Lightner DA. *Tetrahedron: Asymmetry*. 1999; 10:607.
10. McDonagh AF, Boiadjev SE, Lightner DA. *Drug Metab Dispos*. 2008; 36:930. [PubMed: 18299337]
11. McDonagh AF, Lightner DA. *J Med Chem*. 2007; 50:480. [PubMed: 17266200]
12. Lightner DA, McDonagh AF. *J Perinatol*. 2001; 21:S13. [PubMed: 11803409]

13. Brower JO, Lightner DA, McDonagh AF. *Tetrahedron*. 2000; 56:7869.
14. McDonagh, AF.; Lightner, DA. The importance of molecular structure in bilirubin metabolism and excretion. chap 5. In: Bock, KW.; Gerok, W.; Matern, S., editors. *Hepatic Metabolism and Disposition of Endo and Xenobiotics*; Falk Symposium No. 57. The Netherlands: Kluwer, Dordrecht; 1991. p. 47-59.
15. Becker W, Sheldrick WS. *Acta Cryst*. 1978; B34:1298.
16. Pfeiffer WP, Lightner DA. *Tetrahedron Lett*. 1994; 35:9673.
17. Shrout DP, Puzicha G, Lightner DA. *Synthesis*. 1992:328.
18. Trull FR, Person RV, Lightner DA. *J Chem Soc Perkin Trans*. 1997; 2:1241.
19. Chen Q-Q, Ma J-S, Wang C-Q, Liu Y-Y, Yan F, Cheng LJ, Jin S, Falk H. *Monatsh Chem*. 1995; 126:983.
20. Chen, Q-Q, Falk H. *Monatsh Chem*. 1995; 126:1097.
21. Boiadjev SE, Lightner DA. *Synlett*. 1994:777.
22. McMurry JE. *Chem Rev*. 1989; 89:1513.
23. Lenoir D. *Synthesis*. 1989:883.
24. Shrout DP, Lightner DA. *Synthesis*. 1990:1062.
25. Boiadjev SE, Lightner DA. *Org Prep Proced Int*. 2006; 38:347.
26. Thyran T, Lightner DA. *Tetrahedron Lett*. 1995; 36:4345.
27. Paine JB, Dolphin D. *Can J Chem*. 1976; 54:411.
28. Pretsch, E.; Clerc, T.; Serbl, J.; Simon, W. *Tabellen zur Strukturaufklärung organischer Verbindungen*. 3rd edn.. Heidelberg: Springer-Verlag; 1986.
29. Falk H, Flödl H. *Monatsh Chem*. 1985; 116:1177.
30. Falk, H. *The Chemistry of Linear Oligopyrroles and Bile Pigments*. Vienna: Springer-Verlag; 1989.
31. Daroca A, Mercè R, Ribó J, Trull F, Vallès A. *Monatsh Chem*. 1984; 115:357.
32. Fischer H, Adler E. *Hoppe-Seyler's Z Physiol Chem*. 1931; 200:209.
33. Tu B, Ghosh B, Lightner DA. *J Org Chem*. 2003; 68:8950. [PubMed: 14604367]
34. Lightner DA, Crandall DC. *Tetrahedron Lett*. 1973; 21:1799.
35. Nogales D, Anstine DT, Lightner DA. *Tetrahedron*. 1994; 50:8579.
36. Nogales, DF. Ph.D. thesis. Univ. Nevada; 1993. Hydrogen bonding and solution conformation of bilirubin and its analogs.
37. Nogales DF, Ma J-S, Lightner DA. *Tetrahedron*. 1993; 49:2361.
38. Huggins MT, Lightner DA. *Monatsh Chem*. 2001; 132:203.
39. Boiadjev SE, Anstine DT, Lightner DA. *J Am Chem Soc*. 1995; 117:8727.
40. Boiadjev SE, Anstine DT, Maverick E, Lightner DA. *Tetrahedron: Asymmetry*. 1995; 6:2253.
41. Chen Q, Lightner DA. *J Org Chem*. 1998; 63:2665. [PubMed: 11672135]
42. Tipton AK, Lightner DA. *Monatsh Chem*. 1999; 130:425.
43. Huggins MT, Salzameda NT, Lightner DA. *Supramol Chem*. 2011; 23:226.
44. Lightner DA, Gawroński JK, Wijekoon WMD. *J Am Chem Soc*. 1987; 109:6354.
45. Lightner DA, Wijekoon WMD, Zhang MH. *J Biol Chem*. 1988; 263:16669. [PubMed: 3182807]
46. Pu Y-M, McDonagh AF, Lightner DA. *J Am Chem Soc*. 1993; 115:377.
47. Chen Q-Q, Falk H, Murica R. *Monatsh Chem*. 1995; 126:473.
48. Chen QQ, Yan F, Ma JS, Jin S. *Chinese Chem Lett*. 1993; 4:576.
49. Puzicha G, Shrout DP, Lightner DA. *J Heterocycl Chem*. 1990; 27:2117.
50. Nogales D, Anstine DT, Lightner DA. *Tetrahedron*. 1994; 50:8579.
51. McDonagh AF, Lightner DA, Nogales DF, Norona W. *FEBS Lett*. 2001; 506:211. [PubMed: 11602247]
52. Tu B, Ghosh B, Lightner DA. *Monatsh Chem*. 2004; 135:519.
53. Nikitin EN, Lightner DA. *Monatsh Chem*. 2009; 140:97.
54. McDonagh AF. *Monatsh Chem*. 2014; 145:465.

Fig. 1.

(A) Bilirubin-IX α in a porphyrin-like representation, (B) in its intramolecularly hydrogen-bonded planar projection, and (C) in its most stable ridge-tile conformation. (D) Mesobilirubin-XIII α , (E) and (F) homorubins in the porphyrin-like representation and intramolecularly hydrogen-bonded planar projection, respectively. (G) The *b*-homoverdins and (H) dehydro-*b*-homoverdins of this work

(A)

(B)

(C)

Fig. 2.

(A) Intramolecularly hydrogen-bonded minimum energy conformations of homorubins **1** ($n = 2$) and **2** ($n = 3$) as shown in line drawings with dipyrinones in approximately parallel planes. Ball and stick representations of **1** (B) and **2** (C)

Fig. 3.

Planar representations of (10*Z*) and (10*E*) diastereomers of *b*-homoverdins **3** and **4**. CPK molecular models indicate that the *syn-syn* isomers are more capable of intramolecular hydrogen bonding and that the (10*Z*) isomers would likely be more stable than the (10*E*)

(A)

(B)

(C)

(D)

Fig. 4.

Ball and stick representations of (left) (*Z*) and (right) (*E*) *b*-homoverdins **3** (upper) and **4** (lower) in their lowest-energy conformations, as determined by Sybyl molecular mechanics computations. It is evident that the *syn-syn* conformations about the C(9)-C(10) and C(10a)-C(11) bonds allow for intramolecular hydrogen bonding (see Table 9)

Fig. 5.

Planar representations of dehydro-*b*-homoverdins **5** and **6**. The lactam NH to isopyrrole hydrogen bond shown is known to be strong and conformation-stabilizing in biliverdin and related pyrrole compounds [30]

(A)

(B)

(C)

(D)

Fig. 6. Ball and stick representations of (left) *s-cis* and (right) *s-trans* (9Z,10aZ) **5** (upper) and **6** (lower) in their lowest energy conformations, as determined by Sybyl molecular mechanics computations

Scheme 1.

Scheme 2.

Table 1

^{13}C and ^1H NMR chemical shift /ppm assignments^a of homorubin dimethyl esters **1e** and **2e** compared with mesobilirubin-XIII α dimethyl ester (MBRe)

Atom Number ^b	1e		MBRe ^c		2e	
	Carbon	Proton	Carbon	Proton	Carbon	Proton
1, 19	173.2	–	172.4	–	172.0	–
2, 18	135.3	–	130.8	–	133.0	–
3, 17	146.2	–	147.6	–	147.3	–
4, 16	122.6	–	123.0	–	122.3	–
5, 15	99.2	5.85	98.0	5.90	97.8	5.86
6, 14	122.5	–	122.3	–	122.2	–
7, 13	127.5	–	128.2	–	127.4	–
8, 12	117.7	–	119.4	–	120.0	–
9, 11	122.7	–	123.4	–	122.7	–
10, 10a	26.4	3.12	24.0	3.92	26.2	3.12
21, 24	–	10.28	–	9.71	–	10.28
22, 23	–	11.01	–	10.37	–	11.03
21, 18 ¹	9.1	2.12	9.5	1.95	8.1	2.10
31, 17 ¹	17.3	2.32	17.6	2.88	17.2	2.32
32, 17 ²	14.4	1.03	15.3	1.06	14.9	1.02
71, 13 ¹	6.8	1.19	8.5	1.75	9.3	1.20
81, 12 ¹	19.5	2.83	19.7	2.48	25.7	2.52
82, 12 ²	35.5	2.51	34.3	2.46	23.0	1.85
83, 12 ³	173.3	–	173.2	–	32.9	2.41
84, 12 ⁴	–	–	–	–	173.2	–
85, 12 ⁵	51.2	3.72	51.4	3.40	51.1	3.70

^aChemical shifts in ppm downfield from (CH₃)₄Si in CDCl₃, with assignments from gHSQC and gHMBC experiments.

^bSee Fig. 1 for atom numbering. Superscripts refer to the carbons in the pyrrole β -substituent changes, e.g., 2¹ is the first carbon attached to the ring on carbon C(2).

^cData from Ref. [18].

Table 2

^{13}C and ^1H NMR chemical shift /ppm assignments^a of homorubins **1** and **2** compared with those of mesobilirubin-XIII_a (MBR)

Atom Number ^b	1			MBR ^c			2		
	C-13	H ^a	H ^e	C-13	H ^a	H ^e	C-13	H ^a	H ^e
1, 19	172.0	-	-	172.4	-	-	171.9	-	-
2, 18	133.0	-	-	130.8	-	-	132.9	-	-
3, 17	147.2	-	-	147.7	-	-	147.0	-	-
4, 16	122.3	-	-	123.0	-	-	122.1	-	-
5, 15	97.8	5.94	6.00	98.2	5.95	6.05	97.9	5.96	f
6, 14	122.1	-	-	122.4	-	-	122.2	-	-
7, 13	127.6	-	-	128.3	-	-	127.5	-	-
8, 12	119.0	-	-	119.7	-	-	119.8	-	-
9, 11	122.8	-	-	123.4	-	-	122.5	-	-
10, 10a	26.4	2.82	3.34	24.0	3.96	4.08	26.3	2.79	3.01
21, 24	-	9.79	8.59	-	9.77	9.15	-	9.87	9.04
22, 23	-	10.17	10.18	-	10.31	10.58	-	10.15	10.36
OH	-	11.95	13.94	-	11.89	13.64	-	12.03	14.17
2 ¹ , 18 ¹	8.1	1.78	1.86	8.5	1.78	1.86	9.0	1.78	-
3 ¹ , 17 ¹	17.2	2.43	2.45	17.6	2.4	2.48	17.0	2.44	f
3 ² , 17 ²	14.8	1.08	1.10	15.3	1.01	1.12	14.5	1.08	1.20
7 ¹ , 13 ¹	9.3	2.00	2.12	9.6	2.00	2.16	7.8	1.97	1.93
8 ¹ , 12 ¹	19.3	2.53	2.75	19.7	2.43	2.74	25.7	2.21	2.16
8 ² , 12 ²	34.9	2.45	2.86	34.8	1.98	2.84	22.9	1.40	1.29
8 ³ , 12 ³	-	-	-	-	-	-	33.1	2.08	f
8 ⁴ , 12 ⁴	174.1	-	-	174.5	-	-	174.2	-	-
8 ⁵ , 12 ⁵									

^aChemical shifts in ppm downfield from (CH₃)₄Si in (CD₃)₂SO.

^bSee Fig. 1 for atom numbering. Superscripts refer to the carbons in the pyrrole β-substituent chains, e.g., 2¹ is the first carbon attached to the ring on carbon C(2).

^cData from Ref. [13].

d Data from Ref. [18].

e In CDCI₃.

f 2.48–2.60 (12H, m)

Table 3

^{13}C NMR and ^1H NMR spectral data^a from *b*-homoverdin dimethyl esters **3e** and **4e** in $(\text{CD}_3)_2\text{SO}$ and dehydro-*b*-homoverdin dimethyl esters **5e** and **6e** in CDCl_3

Position ^b	Carbon	<i>b</i> -Homoverdin			Dehydro- <i>b</i> -homoverdin			<i>b</i> -Homoverdin			
		3e		4e		5e		6e		Chen et al.	
		¹³ C	¹ H	¹³ C	¹ H	¹³ C	¹ H	¹³ C	¹ H		
1, 19	C=O	173.5		173.2		172.7		173.8		172.2	
2, 18	–C=	123.4		123.5		147.4		147.5		148.0	
3, 17	–C=	146.8		147.1		146.1		146.3		146.7	
4, 16	–C=	129.2		129.3		130.6		130.5		130.7	
5, 15	–CH=	95.8	5.9	96.8	5.9	95.5	5.8	95.5	5.8	95.5	5.68
6, 14	–C=	125.1		123.8		170.5		170.6		171.0	
7, 13	–C=	125.2		123.8		138.0		137.5		138.8	
8, 12	–C=	126.0		125.2		142.3		142.3		142.6	
9, 11	–C=	131.5		132.2		159.8		160.2		160.4	
10, 10a	CH=	113.1	6.8	113.5	6.8	125.2	7.8	125.3	7.8	125.8	7.76
2 ¹ /18 ¹	CH ₃	7.4	1.8	8.1	1.8	8.5	1.8	8.6	2.1	9.8	1.95
3 ¹ /17 ¹	CH ₂	18.0	2.3	17.2	2.3	17.7	2.3	17.7	2.5	17.7	2.60
3 ² /17 ²	CH ₃	15.0	1.1	14.8	1.2	14.3	1.0	14.4	1.2	14.2	1.18
7 ¹ /13 ¹	CH ₃	9.7	2.1	9.1	2.1	10.1	2.1	10.1	2.0	8.5	2.10
8 ¹ /12 ¹	CH ₂	20.2	2.8	25.8	2.5	19.9	2.5	23.8	2.8	20.1	2.96
8 ² /12 ²	CH ₂	36.1	2.5	23.1	2.8	35.1	2.4	26.1	1.9	35.3	3.08
8 ³ /12 ³	CH ₂	–	–	32.7	2.4	–	–	33.3	2.4	–	–
	CO ₂ H	173.2		172.1		172.2		172.3		172.7	
	OCH ₃	51.8	3.6	51.1	3.7	51.8	3.6	51.7	3.6	51.7	
	L-NH		10.2		10.2		10.5		10.5		<i>d</i>
	P-NH		10.3		10.3		–		–		<i>d</i>

^a Determined for $\sim 1 \times 10^{-3}$ M concentrations.

^b For numbering see Fig. 1.

^{13}C NMR in pyridine- d_5 ; ^1H NMR in CDCl_3 .

d Not reported.

Table 4

Comparisons of UV-Vis long wavelength absorption 1 spectral data^a of homorubins **1** and **2** with mesobilirubin-XIII_α (MBR-XIII)

Solvent	Homorubin 1		Homorubin 2		MBR-XIII ^b	
	λ^{max}	ϵ^{max}	λ^{max}	ϵ^{max}	λ^{max}	ϵ^{max}
Benzene	423	61,210	420	51,800	433	55,400
CHCl ₃	424	57,000	421	51,700	431	57,800
Acetone	407	50,100	411	49,500	426	55,400
Acetonitrile	418	57,100	407	50,300	423	55,000
CH ₃ OH	428	65,100	417	55,300	426	50,700
					401 ^{sh}	43,100
(CH ₃) ₂ SO	425	65,700	405	56,500	426	57,000
	—	—	—	—	397 ^{sh}	49,100

^a At room temperature for 1×10^{-5} M solutions; λ^{max} / nm, ϵ^{max} / dm³ mol⁻¹ cm⁻¹.

^b Data were taken from Ref. [18]

Table 5

Solvent-dependence of the UV-Vis spectral data^a of *b*-homoverdins **3** and **4**, their dimethyl esters (**3e** and **4e**), and dehydro-*b*-homoverdin dimethyl esters (**5e** and **6e**)

	Benzene	CHCl ₃	(CH ₃) ₂ CO	CH ₃ CN	CH ₃ OH	(CH ₃) ₂ SO
3	538 (25,600)	544 (25,200)	533 (24,000)	531 (26,900)	537 (32,800)	533 (27,000)
4	540 (18,600)	545 (22,300)	532 (17,800)	533 (16,900)	539 (32,800)	534 (24,500)
3e	530 (33,500) 402 (31,800)	534 (33,600) 400 (26,800)	522 (36,100) 395 (25,000)	520 (34,000) 395 (21,100)	533 (38,300) 400 (19,400)	533 (36,500) 402 (20,600)
5e	534 (41,800) 399 (45,900)	533 (35,200) 395 (44,000)	521 (38,300) 390 (45,400)	530 (26,100) 398 (37,000)	527 (30,300) 393 (38,200)	524 (46,200) 387 (44,300)
4e	536 (32,700)	537 (31,400)	520 (36,500)	519 (37,200)	540 (38,000)	538 (37,100)
6e	545 (37,600)	541 (34,500)	534 (35,100)	532 (36,100)	530 (35,000)	545 (36,200)

^a 1×10^{-5} M concentration. The molar absorption coefficient ($\epsilon^{\max} / \text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$) are shown in parentheses below $\lambda^{\max} / \text{nm}$.

Table 6

R_f values from TLC on silica gel with 1 CH_2Cl_2 - CH_3OH (95:5 by vol) eluent, and reversed phase HPLC retention times of the bilirubin analogs. Flow rate = 1 cm^3/min

Compound	R_f	Retention times/min
Homorubin 1	0.63	12.83
Homorubin 2	0.92	15.37
Mesobilirubin	0.89	14.85

Table 7

Comparison of the lactam and pyrrole N-H chemical shifts^a of homorubins **1** and **2** in CDCl₃ and (CD₃)₂SO with those of mesobilirubin-XIII_α

Compound	NH chemical shift in CDCl ₃ /ppm		NH chemical shift in (CD ₃) ₂ SO/ppm	
	Lactam	Pyrrole	Lactam	Pyrrole NH
1	10.18	8.59	9.79	10.16
2	10.36	9.04	9.87	10.15
Mesobilirubin	10.58	9.15	9.72	10.31

^aRun at 2×10^{-4} M pigment concentration at 25 °C.

Table 8

Comparison of the induced circular dichroism 1 and UV-Vis spectra for homorubins **1** and **2** and mesobilirubin-XIII α in (A) phosphate buffer at pH 7.4 and a 2:1 pigment:HSA molar ratio, and in (B) CHCl $_3$ in the presence of quinine (pigment:quinine molar ratio: 1:300)

Compound	Circular Dichroism				UV-Vis	
	$\epsilon^{\max}(\lambda_1)^b$	λ at $\epsilon=0^b$	$\epsilon^{\max}(\lambda_2)^b$		$\epsilon^{\max} \lambda^b$	
(A) Homorubin 1	+94 (443)	414	-80 (395)		52,010 (411)	
Homorubin 2	+5.5 (445)	405	-5.7 (392)		47,400 (434)	
Mesobilirubin-XIII α ^b	+45 (436)	410	-42 (390)		46,600 (436)	
(B) Homorubin 1	~0	-	~0		44,720 (420)	
Homorubin 2	~0	-	~0		63,300 (426)	
Mesobilirubin-XIII α ^b	+121 (434)	409	-80 (395)			

^aFor 1×10^{-5} M pigment solutions in a 1 cm pathlength cuvette.

^b ϵ and $\epsilon^{\max}$ / dm 3 mol $^{-1}$ cm $^{-1}$ and $\lambda^{\max}$ / nm. Data taken from Ref. [44]

Table 9

Comparison of computed torsion angles and nonbonded distances^a of 1 the (10*Z*) and (10*E*) *b*-homoverdins **3** and **4** and mesobilirubin XIII_α (MBR) in their most stable conformations

Torsion angles/°					Distances/Å			H-Bond angles/°				
n	φ ₁	φ ₂	φ ₃	ψ ₁	ψ ₂	a	b	c	OH...O	N(22)-H...O	N(21)-H...O	
3 (Z)	2	42	2	38	4	17	1.56	1.52	1.73	165	154	152
3 (E)	2	43	4	29	25	28	1.57	1.71	1.60	164	158	132
4 (Z)	3	39	2	27	16	7	1.57	1.69	1.60	170	166	143
4 (E)	3	11	2	2	12	17	1.52	1.61	1.66	168	165	148
MBR	2	64	–	64	17	17	1.53	1.59	1.56	170	163	154

^a From Sybyl vers. 7.1 run on an SGI workstation. The small ψ₁, ψ₂ torsion angles indicate that the relevant nitrogens are nearly *syn*-periplanar.

Comparison of computed torsion angles and nonbonded distances^a of dehydro-*b*-homoverdins **5** and **6** in their most stable (9*Z*,10*aZ*) conformations

	Torsion angles/ $^{\circ}$				Distances/ $\text{\AA}$		H-Bond angles/ $^{\circ}$		
	n	ϕ_1	ψ_1	ψ_2	a	b	OH...O	N(22)-H...O	N(21)-H...N(22)
5 <i>s-cis</i>	2	35	3	0	1.62	3.24	140	102	123
5 <i>s-trans</i>	2	185	2	0	1.51	2.99	172	124	123
6 <i>s-cis</i>	3	28	5	1	1.51	2.90	168	112	123
6 <i>s-trans</i>	3	182	20	1	1.51	1.89	169	121	118

^a From Sybyl vers. 7.1 run on an SGI workstation. The $\sim 0^\circ$ torsion angles are those where the relevant nitrogens are *syn*-periplanar.