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(m.n)-Homorubins. Syntheses and Structures

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Abstract

Five new homorubin analogs of bilirubin with their two dipyrinone components conjoined to $(\text{CH}_2)_2$, $(\text{CH}_2)_3$, and $(\text{CH}_2)_4$ units were synthesized with propionic acid chains shortened to acetic and elongated to butyric, and examined by spectroscopy and molecular mechanics computations for an ability to form conformation-determining hydrogen bonds. With m designating the number of conjoining CH_2 units and n indicating the number of CH_2 units of the alkanoic acid chains of (m.n)-homorubins, (2.1), (3.2), (4.2), and (4.3) homorubins were prepared and compared with previously synthesized (2.2) and (2.3), which adopt intramolecularly hydrogen bonded conformations in CHCl_3 .

Keywords

Bilirubinoids; Synthesis; Molecular structure; Conformational analysis

Introduction

Bilirubin (Fig. 1A) is the yellow pigment of human bile and jaundice [1], produced copiously as the end-product of porphyrin metabolism [2]. Its most stable conformation in the crystal [1, 3–5] and in solution [1, 6–9] is one where the two dipyrinone chromophores are rotated about C(10) so as to bring each dipyrinone into hydrogen bonding with one of its two propionic acid groups (Fig. 1B). The analogs of bilirubin with vinyl groups reduced to ethyls, e.g., mesobilirubin-XIII α (Fig. 1C, where m = 1, n = 2), also adopt an intramolecularly hydrogen-bonded structure [9, 10] and were found to exhibit similar solution and metabolic properties [11, 12]. In order to learn whether intramolecular hydrogen bonding might persist in a centrally homologated mesobilirubin, yet remain stable, we linked the two dipyrinones to two CH_2 connector groups. We communicated [13] our synthesis of (2.2)-homorubin (Fig. 1C, where m = n = 2) and compared its properties to those of mesobilirubin-XIII α . The work indicated the presence of bilirubin-like intramolecular hydrogen bonding and a hepatobiliary metabolism similar to that of bilirubin and mesobilirubin-XIII α . More recently we reported on a (2.3)-homorubin, with butyric

acids replacing propionic, and this too, proved to be intramolecularly hydrogen bonded, although its shape was modified to accommodate the longer butyric acid [14].

Prompted by our first communication on homorubin [13], we undertook the syntheses and analyses of a series of (m,n)-homorubins (Fig. 1C), where m varied between 1 and 4 and n varied between 1 and 3. At first, we conducted a series of conformational analysis studies by molecular dynamics using Sybyl to learn whether the carboxylic acids could engage in intramolecular hydrogen bonding to the dipyrinones and to estimate the stabilization energies and number of effective hydrogen bonds (Table 1). We found the predictions of Table 1 for (2,2)- and (2,3)-homorubins to correlate nicely with their solution and spectroscopic properties and thus extended the investigations to other (m,n)-homorubins, notably (2,1), (3,2), (4,1), (4,2), and (4,3), whose syntheses and analyses are presented in the following. We were especially interested in pairs of the same molecular formulas, (2,2) and (3,1); (3,3) and (4,2); and the triplet (2,3), (3,2), and (4,1). Unfortunately, we were unable to prepare the (3,1) and (3,3)-homorubins and thus report only the (3,2) of the m = 3 set. The (2,2) and (2,3)-homorubins are known [13, 14]; the (1,2) is mesobilirubin-XIIIa, also known [15].

Results and Discussion

Homorubin synthesis aspects

The syntheses of (m,n)-homorubins, where m is an even integer proved to be conducted best by the “1 + 2 + 1” route [16], where a dipyrrole core was expanded to the desired tetrapyrrole by the addition of two equivalents of a reactive monopyrrole. This approach worked successfully in the synthesis of the first homorubin, where m = 2, with propionic acid chains at the usual locations, C(8) and C(12) [13] – and recently for the (2,3)-homorubin with butyric acid chains [14].

(4,n)-Homorubins

It also turned out to work well in the syntheses of the (4,1), (4,2), and (4,3)-homorubins, **4-1**, **4-2**, and **4-3**, which were all synthesized (Scheme 1) following saponification of their dimethyl esters (**4-1e**, **4-2e**, and **4-3e**) prepared by condensing the well-known 5-bromomethylene 2-pyrrolinone (**12**) [17, 18] with a 1,4-dipyrrolylbutane core (**5-n**). Under the reaction conditions, **5-n** underwent decarboxylation of both pyrrole α -CO₂H groups to give reactive α,α -dipyrroles, which coupled with **12** in standard fashion [17–19]. It was thought that the most practical way to introduce the 1,4-disubstituted butane unit of **5-n** would be as a diacetylene unit (as in **6-ne**) formed by Pd(0)-catalyzed coupling of the two mono-acetylenes. The target mono-acetylenes would thus be **7-n** for which we envisioned introduction of the acetylene by a Sonogashira reaction [20–22] of trimethylsilyl acetylene with an α -iodopyrrole (**9-n**). The α -iodopyrroles **10-n** were prepared in four steps from simple, acyclic starting materials: diethyl oximinomalonate and the known acyl-oxo-esters **12-n** [23] to afford mono-pyrrole diesters **11-n**, prepared in connection with earlier synthetic work [23].

The α -CH₃ of **11-n** was converted to α -I [24] first by perchlorination with SO₂Cl₂, followed by hydrolysis of the newly formed α -CCl₃ to convert it to α -CO₂H (**10-n**). The last was converted to α -I upon treatment [24] with KI/I₂ ethanol (**10-1**) or methanol (**10-2**, **10-3**). The resultant α -I pyrrole **9-n** formed in good yield and served as the entry to diacetylenic dipyrrole **6-ne**. An acetylene group was substituted smoothly and in high yield for α -I using trimethylsilyl (TMS) acetylene in the presence of bis(triphenylphosphine)palladium(II) chloride plus copper(I) iodide [20–22, 25]. After initial attempts to desilylate **8-n** using 1 M aq. NaOH gave very poor yields, the TMS acetylene group of **8-n** was deprotected by adding anhydr. K₂CO₃ in anhydrous ethanol to a solution of **8-n** in dry tetrahydrofuran (THF). Thus, **7-n** was formed in good yield, and it was self-coupled to **6-ne** using tetrakis(triphenylphosphine)palladium(0) and Cu(I) catalyst in the presence of chloroacetone and triethylamine [26]. Hydrogenation of diacetylenes **6-1e** and **6-2e** over 5% Pd(C) catalyst in ethyl acetate at 1 bar gave nearly quantitative yields of the target 1,4-dipyrrolbutane esters **5-1e** and **5-2e**. Attempts to hydrogenate **6-3e** under the same conditions, or at 2.8 bar were unsuccessful; however, success was achieved using PtO₂ catalyst at 1 bar over 24 h. Saponification of **5-ne** in aqueous ethanolic NaOH, followed by removal of the ethanol and acidification with 20% HNO₃ produced white precipitates of **5-n**, which were collected and dried. Acids **4** tended to be less stable than their ($m = 2$) analogs, with their colors changing to pink or brown when exposed to air for more than a few hours. They were converted to tetrapyrrole esters **4-ne** by suspending them in CH₃OH, bringing the suspension to reflux, and adding two equivalents of 5-bromomethyl-2-pyrrolinone (**12**). After 3 h at reflux, the product **4-ne** started to precipitate. Surprisingly and unexpectedly, all of the homorubin esters **4-ne** are very insoluble in many organic solvents. For example, heat and ultrasonication are required to dissolve them completely in dimethyl sulfoxide (DMSO) for characterization by nuclear magnetic resonance (NMR) spectroscopy. After sitting a few days in DMSO, they begin to precipitate. The best purification method for the esters **4-ne** was to dissolve them in hot dimethyl formamide (DMF) (~110 °C) and allow them to precipitate upon cooling. The precipitates were filtered, washed with ethanol to remove any traces of DMF, then washed again with CH₂Cl₂ to remove any additional impurities that may have been in the precipitate. These solubility properties of **4-ne** are very peculiar compared to the shorter bridged dimethyl analogs where ($m = 1$) and ($m = 2$). One might assume that by interspersing more CH₂ units ($m = 4$) between the dipyrrolinone units in **4-ne**, the compounds would be more soluble in organic compounds, but that is not the case. Strangely, during the saponification of **4-ne** to **4-n**, although esters **4-ne** could only be suspended (and not dissolved) in hot THF-CH₃OH, upon addition of aq. NaOH they go immediately into solution. After cooling, acidification led to precipitated **4-n** in respectable yields.

(3.n)-Homorubins

The synthesis of a $m = 3$ homorubin, e.g., a three-carbon homolog of mesobilirubin-XIIIa, was initially intended to follow a “1 + 2 + 1” route similar to that used for the $m = 2$ and $m = 4$ homorubins. However, attempts to form a 1,3-bis-pyrrolpropane central unit failed. These included initially attractive routes toward the synthesis of the (3.1)-homorubin analog of **4-1e**. However, as outlined in Scheme 2 (1), though the conversion of iodopyrrole **9-1**

(Scheme 1) to its deiodinated α -H analog proceeded smoothly, as did a vinylogous Vilsmeier reaction to produce the β -(α -pyrrolyl)-acrolein, the last failed to condense with the α -H pyrrole. In Scheme 2 (2) the β -(α -pyrrolyl) acrolein formed in (1) could be reduced to the corresponding allylic alcohol, but as such or as its tosylate failed to condense with the α -H pyrrole precursor. Similar failures were encountered with the C=C reduced analogs. In Scheme 2 (3), we found an unacceptably low yield (~10%) of the final nitro-dipyrrolylpropene product. In Scheme 2 (4), although the pyrrole α -aldehyde could be condensed smoothly with malonic acid, neither the resultant unsaturated diacid nor its hydrogenation product could be smoothly decarboxylated in: (i) ethanol-conc. HCl (1:1 by vol.) at reflux; (ii) aniline or piperidine at reflux; (iii) molten KOAc-NaOAc; or (iv) by heating to 230 °C.

Therefore, we resorted to a “2 + 2” route (Scheme 3) described by Chen et al. [27] to prepare a conjugated bis-dipyrinone propene. As modified to neoxanthobilirubinic acid methyl ester [28], reaction of **14** with dimethylaminoacrolein yielded the vinylogous Vilsmeier product **15** in good yield. Condensation of **14** and **15** in acetic acid-HBr afforded the desired product **13**. Reduction of **13** first with NaBH₄ to reduce the azafulvene unit, followed by catalytic hydrogenation of the residual C=C afforded the m = 3, n = 2 homorubin dimethyl ester (**3-2e**), from which the (3.2)-homorubin (**3-2**) was obtained after saponification.

Neoxanthobilirubinic acid methyl ester (**14**) was synthesized from **17** [29] and **18** [30], which were prepared from small molecules, as outlined in Scheme 3. Nitroethane and propionaldehyde were carried through five steps, involving the Barton-Zard pyrrole reaction to the known 4-ethyl-3-methyl-3-pyrrolin-2-one (**17**), as described previously [29]. Similarly, as described earlier, succinic anhydride and acetylacetone were carried through a series of four steps to produce pyrrole diester **19** [18]. The pyrrole α -CH₃ was oxidized to pyrrole α -aldehyde **18** [30] by ceric ammonium nitrate [31, 32]. Condensation of **17** with **18** in 4 M KOH-CH₃OH yielded dipyrinone diacid **16** [33], which was smoothly α -decarboxylated and esterified in CH₃OH-H₂SO₄ to afford dipyrinone **14** [33] in 59% yield from **17** and **18**. The synthesis of tetrapyrrole **13** required the dipyrinone coupling partner **15**, which was prepared from **14** in good yield by reaction with dimethylaminoacrolein. Dipyrinones **14** and **15** were smoothly condensed to form the conjugated 1,3-bis-dipyrinone propene **13** in 86% yield, and **13** was reduced to **3-2e** as described above. Saponification of **3-2e** yielded the desired **3-2**.

(2.1)-Homorubins

Reverting to the “1 + 2 + 1” approach to linear tetrapyrrole synthesis, we employed a route similar to that used for preparing (2.2)-homorubin [13, 14] and (2.3)-homorubin [14]. Here and in the (2.2)- and (2.3)-homorubin, the required center dipyrrole sections differed only by the length of the carboxylic acid chain: acetic vs. the propionic and butyric of earlier work [13, 14]. Thus, as shown in Scheme 4, the α -CH₃ of the known mono-pyrrole diester **11-1** [23] was converted to the α -CHO of **22** in 66% yield by oxidation using ceric ammonium nitrate [31, 32], and **22** was coupled to afford dipyrrolethene **21** in 46% yield by the McMurry coupling procedure [13, 14, 34] using Ti⁰. The central C=C of **21** in ethyl acetate

was smoothly hydrogenated ($\text{H}_2/\text{Pd}(\text{C})$) to give a 99% yield of dipyrrolethane tetra-ester **20e**, which was saponified in ethanolic NaOH to its tetra-acid **20** in 99% yield. The last, when dissolved in refluxing CH_3OH in the presence of 5-bromoethylene-3-pyrrolin-2-one (**12**), underwent decarboxylation of the $\alpha\text{-CO}_2\text{H}$ groups and coupling to **1** to afford tetrapyrrole dimethyl ester **2-1e** in 51% yield, which was saponified to the desired $m = 2$, $n = 1$ homorubin (**2-1**) in 52% yield.

Conformational analysis

Bilirubin-IX α (Fig. 1A, B) and mesobilirubin-XIII α (Fig. 1C) preferentially adopt an intramolecularly hydrogen-bonded conformation. This conformation, shaped like a half-open book (Fig. 2A), named “ridge-tile” [3], which minimizes destabilizing non-bonded steric interactions, is more stable than all others, and as such it plays a dominating role in the pigment’s physico-chemical properties and metabolism [1, 11–15, 29]. In our earlier studies with (2.2)- and (2.3)-homorubins, we investigated how homologating the central CH_2 unit of mesobilirubin-XIII α affects (i) the ability of the pigment to engage in intramolecular hydrogen bonding and (ii) the most stable shape of the (2.2)-homorubin molecule [13], which was found to adopt a layered conformation with intramolecular hydrogen bonding. More recently, we showed that even with homologating the propionic acid chains to butyric, this pigment also strongly favors intramolecular hydrogen bonding and also with more of a layered shape [14]. These studies led to an investigation of other homorubins, for which Sybyl molecular dynamics calculations predicted that while six intramolecular hydrogen bonds persisted in the (2.1), (2.2), (2.3), (3.2), and (4.3)-homorubins, four hydrogen bonds were engaged in the (4.1) and (4.2)-homorubins (Table 1). The energy-minimum molecular geometry, defined by torsion angles (A–F) and dihedral angles (θ) in and about the dipyrinone units (Fig. 2B) indicates a quasi-ridge-tile shape for the (2.1) and (4.1)-homorubins (Fig. 2B) and a different geometry for the (2.2), (2.3), (3.2), (4.2), and (4.3)-homorubins, where the dipyrinones tend to lie in parallel planes (Fig. 2C). The shapes of the energy-minimized molecules are suggested by their Ball and Stick representations in Fig. 3. It should be noted that while all of the conformations are stabilized by intramolecular hydrogen bonding, two do not have a full complement of six hydrogen bonds. The carboxyl OH distance to the lactam $\text{C}=\text{O}$ is predicted to be too long to permit a hydrogen bond in the (4.2)-homorubin, as is the pyrrole N-H to carboxy $\text{C}=\text{O}$ hydrogen bond of the (4.1)-homorubin (Table 2).

Structural aspects and ^{13}C NMR

The assigned constitutional structures of the homorubins follow from the method of synthesis and are confirmed by their ^{13}C NMR spectral data, as obtained in $(\text{CD}_3)_2\text{SO}$ and shown in Tables 3–5. Table 3 shows good correlation for the ^{13}C NMR chemical shifts of homorubins with acetic acid chains (**2-1** and **4-1**) to those of the previously studied mesobilirubin-XIII α with propionic acids replaced by acetic acids [15]. In like manner, Table 4 compares ^{13}C NMR chemical shifts for the pigments with propionic acid chains, (2.2), (3.2), and (4.2)-homorubins with those of mesobilirubin-XIII α [15], again finding good correlation. Finally, Table 5 shows a good correlation for the ^{13}C NMR chemical shifts of the rubins with butyric acid chains, $n = 3$ mesobilirubin-XIII α , and the (2.3) and (4.3)-

homorubins. The data clearly show strong similarities across the range of homorubin structures possessing the same alkanolic acid chain length.

Hydrogen bonding aspects and ^1H NMR

Much concerning hydrogen bonding can be learned from an examination of bilirubinoid pigment N-H chemical shifts in CDCl_3 , a solvent in which bilirubin and mesobilirubin retain the intramolecular hydrogen bonding found in the crystal [3–5, 10]. In CDCl_3 , the ^1H NMR chemical shifts of lactam and pyrrole NHs typically lie between 7 and 8 ppm in the absence of hydrogen bonding and undergo considerable deshielding when hydrogen bonded [35, 36]. Previous studies of bilirubinoid pigments showed a deshielding to ~10.5 ppm (lactam) and ~9 ppm (pyrrole) when the dipyrinone and carboxylic acid groups were intramolecularly hydrogen bonded in CDCl_3 [37]. Whereas, when intermolecular hydrogen bonding prevails between dipyrinones, the chemical shifts are found near 11 ppm (lactam) and 10 ppm (pyrrole) in CDCl_3 [36, 37]. In contrast, in $(\text{CD}_3)_2\text{SO}$ solvent, the ^1H NMR NH chemical shifts generally lie 9.5–9.9 ppm (lactam) and 10.1–10.3 ppm (pyrrole). Kaplan and Navon [38] showed that in $(\text{CD}_3)_2\text{SO}$ solvent molecules are bound up in the intramolecular hydrogen bonding matrix. Thus, an examination of the NH ^1H NMR chemical shifts in CDCl_3 is an excellent way of investigating intramolecular hydrogen bonding (in a non-polar solvent) [6]. The important indicator in CDCl_3 is a pyrrole N-H chemical shift near 9 ppm, more shielded than expected because the N-H lies over or near the face of a second pyrrole ring and experiences diamagnetic anisotropic shielding by the pyrrole's π -system. This implies a conformation for the pigment, and in bilirubin and mesobilirubin-XIII α , such a conformation is the same as that seen in the crystal [3–5, 10], or that shown in Fig. 1A – and well established in CDCl_3 by sophisticated NMR spectroscopic analysis [6–8].

The ^1H NMR chemical shifts of the lactam and pyrrole N-Hs are given in Table 6 along with data from mesobilirubin-XIII α . In CDCl_3 , the deshielded lactam N-H chemical shifts, ranging from 9.6–10.6 ppm, are characteristic of hydrogen bonding and consistent with intramolecular hydrogen bonding, as one might deduce. Similarly, from the pyrrole N-H chemical shifts in CDCl_3 . A rather large range of pyrrole N-H chemical shifts, from 8.01 (3-2) to 9.04 (2-3) ppm is seen. The more shielded values, e.g., those < 9 ppm, might be attributed to either more effective diamagnetic shielding and/or less effective hydrogen bonding of the pyrrole N-H in 2-2, 3-2, 1-2, and 1-3. The first rationale is supported by the energy- minimized intramolecularly hydrogen-bonded structures of Fig. 3.

An unusual behavior was observed in the ^1H NMR spectrum of the (3.2)-homorubin (3-2) that had not been observed previously for other bilirubin analogs. When the ^1H NMR was determined in CDCl_3 at 25 °C, two additional and unexpected resonances were seen in the N-H chemical shift region of the spectrum (Fig. 4). Their chemical shifts (10.92 ppm and 10.17 ppm, Table 6) correspond to lactam and pyrrole N-Hs, respectively, and are characteristic of *intermolecular* hydrogen-bonded dipyrinones [36, 39]. Upon heating, these signals disappear, indicating that the secondary structure which gave rise to them is not as dynamically stable as the structure that provides the other resonances. Upon cooling, the reverse occurred and the resonances' "new resonances" reappeared. Therefore, one may

venture to assume that some sort of limited intermolecular interaction occurs in non-polar solvents.

Solubility and chromatographic considerations

Investigation of the different solubility, chromatographic, and spectral properties gives insight into whether the homorubin analogs display intramolecular hydrogen bonding, as in mesobilirubin. The first two methods include solubility behavior and thin layer chromatography. Mesobilirubin is soluble in chloroform, indicating that it is somewhat non-polar, consistent with intramolecular hydrogen bonding. (2.2)-Homorubin (**2-2**) seems to have solubility properties similar to those of mesobilirubin, though slightly less soluble in non-polar solvents, consistent with intramolecular hydrogen bonding. The (2.3)- and (3.2)-homorubins (**2-3** and **3-2**) are also somewhat soluble in CHCl_3 to the extent that a reasonable ^1H NMR spectrum could then be obtained for each species. In contrast, the (2.1), (4.1), (4.2), and (4.3)-homorubins are less soluble in non-polar solvents, thus indicating increased polarity with the possibility of less effective intramolecular hydrogen bonding.

Thin layer chromatography (TLC) and high performance liquid chromatographic (HPLC) behavior provided further insight into pigment polarity and thus intramolecular hydrogen bonding. As intramolecular hydrogen bonding weakens or is absent, the pigment's polarity increases. In TLC, increased polarity is seen by slower movement (smaller R_f values) on silica gel; in reversed phase HPLC, it is seen in shorter retention times. The chromatographic behavior of the homorubins of this work relative to that of mesobilirubin- $\text{XIII}\alpha$ is displayed in Table 7. From the HPLC retention times, it would appear that the (3.2), (4.1), and (4.2)-homorubins, especially the last, would appear to be more polar than the others, which are more like mesobilirubin in chromatographic behavior – and probably engaged in more effective intramolecular hydrogen bonding. In regard to polarity (and the effectiveness of intramolecular hydrogen bonding), the TLC behavior is in qualitative agreement.

The dimethyl esters present a somewhat different solubility picture. Although the structures of the various homorubin dimethyl esters are similar, they display significant differences in their solubility properties. It might be expected that, with their longer saturated hydrocarbon chain between the dipyrinone units, the (4.n)-tris-homorubins should be more soluble in organic solvents than the corresponding (2.n) and (3.n)-homorubins. However, it turns out that the tris-homologs are not very soluble in many organic solvents, which raised difficulties in their purification. The only purification method found was to dissolve them in hot dimethyl formamide, cool to room temperature, collect the precipitate, and wash it with 95% ethanol. Unfortunately, due to their poor solubility properties in CDCl_3 , NMR data could not be obtained in this solvent. In order to obtain NMR data, the tris-homorubin esters (**4-1**, **4-2**, and **4-3**) were dissolved by sonication in warm $(\text{CD}_3)_2\text{SO}$. Yet after standing in the NMR tube for more than a day, they would precipitate.

The solubility properties of the (2.1)-homorubin dimethyl ester (**2-1e**) permitted purification by washing with ethyl acetate or acetone. Its poor solubility properties mirror those of the mesobilirubin analog with acetic acid chains replacing propionic [15]. Apparently, poor solubility properties are associated with the short acetic acid ester side chain. Similar

properties were observed in the acetic ester ethene (**21**) and butadiyne (**5-1e**) diester precursors. In contrast, (2.2), (2.3), and (3.2)-homorubin dimethyl esters (**2-2e**, **2-3e**, and **3-2e**) were quite soluble in many organic solvents such as ethyl acetate, CH₂Cl₂, and (CH₃)₂SO. Diester **3-2e** seemed to be just as soluble in these organic solvents as the (2.2) and 2.3) homorubin dimethyl esters. Because the solubility properties are so much worse for the (4.1) analogs than the (2.n) analogs, one might expect that the (3.2)-homorubin **3-2e** would exhibit solubility properties between those of the (2.n) and (4.n) homologs; however that was not observed.

UV-Vis spectral aspects

Further evidence of intramolecular hydrogen bonding in the alkanolic acid homologs comes from solvent-dependent UV-Vis spectra. A noticeable change in the UV-Vis spectrum when switching from a non-polar to a polar solvent unusually indicates a change in the conformation of the molecule [1, 9]. Although this may sound confusing, mesobilirubin does not show any significant shift in absorbance when changing from a non-polar to a polar solvent. Since mesobilirubin is known from NMR studies to adopt the intramolecularly hydrogen-bonded conformation in CDCl₃ solvent one might expect to see a significant change in the UV-Vis absorbance when changed from a non-polar to a polar solvent. However, the absorbance does not vary significantly when changing solvents; thus, it may be argued that mesobilirubin prefers the folded conformation (but not necessarily hydrogen bonded) regardless of the solvent used. Therefore, the UV-Vis absorption properties of the homorubin analogs were measured in a variety of solvents (Tables 8–10) in order to observe any solvent dependence of the conformation.

Table 8 includes data for homorubins with acetic acid chains ($n = 1$) and dipyrinones linked by CH₂ units varying from one to four ($m = 1, 2, 4$). All examples exhibit very similar ϵ_{max} values, but λ_{max} remains fairly constant for the $m = 1, n = 1$ rubin suggesting little solvent influence on its conformation. Unlike that of **2-1**, where λ_{max} varies more with solvent polarity, the data for **4-1** suggest that the pigments adopt conformations where the dipyrinones act independently, viz., not part of or only in a weak exciton system. In Table 9, comparison is made for homorubins with propionic acid chains ($n = 2$) and CH₂ linkers varying between $m = 1$ to $m = 4$. As the well-studied exciton of mesobilirubin-XIII α (MBR-XIII α , $n = 1, n = 2$) [1, 41], there is little variation in λ_{max} for **2-2** and **3-2** with these three apparently adopting conformations where the chromophores may interact. In contrast, **4-2** behaves like **4-1** with two non-interacting or only weakly interactive dipyrinone chromophores. In Table 10, where the pigments have butyric acid chains ($n = 3$) and vary by the number of CH₂ linkers ($m = 1, 2, 4$), again where $m = 1$, the pigment MBR-XIII α behaves like a molecular exciton (as in its analogs of Tables 8 and 9), whereas, **2-3** and **4-3** seem more likely to adopt conformations where the dipyrinone chromophores might interact only in nonpolar chloroform and benzene.

Circular dichroism aspects

When a molecule absorbs UV-visible left and right circularly polarized light unequally, it exhibits circular dichroism (CD). From CD measurements one can often deduce the handedness or stereochemistry of the molecule. In certain cases, achiral molecules or even

racemic mixtures can be complexed with chiral molecules, and an induced circular dichroism results [1, 40]. An example of the latter can be seen with bilirubin. Since bilirubin exists as two interconverting conformation enantiomers (Fig. 2A) it is a racemic mixture in isotropic media and does not show CD. However, chirality can be induced when it binds to a chiral molecule such as quinine [41] or the protein human serum albumin (HSA) [42, 43], giving rise to an induced CD (ICD) and a bisignate curve. The observed bisignate CD comes from exciton coupling of two electric dipole transitions [1, 41]: one from each of the pigment's twin dipyrnone chromophores. In the case of mesobilirubin, from the long wavelength UV-Vis excitation near 420 nm [37, 40]. For the homorubins, ICD experiments were conducted using HSA and quinine as the chiral complexing agents, with the data shown in Tables 11 and 12.

The presence of bisignate curves indicates that the homorubins have fixed conformations when bound to HSA. The results show that the $m = 2$ homorubins have similar binding characteristics to HSA as mesobilirubin, as all exhibit positive exciton chirality. The remaining analogs also have the ability to bind HSA, but the manner in which they bind cannot be determined. The ICD data for all of the homorubins of this work are far weaker in intensity than that observed for mesobilirubin-XIII α [37] or for bilirubin itself [41] – the unique exception being that of (2.2)-homorubin (**2-2**). Whether the weak ICDs reflect poor enantio-selection or poor binding (unlikely in the case of HSA) cannot be determined at present.

With quinine as a chiral complexing agent, the data of Table 12 indicate the previously noted Cotton effect sign reversals for mesobilirubin-XIII α relative to those seen (Table 11) in aqueous buffered albumin, the same (albeit weak) exciton chirality for the (4.n)-homorubins. Curiously, a reversed and strong exciton chirality is seen for the (2.1)-homorubin – whereas the (2.2)- and (2.3)-homorubins exhibit barely detectable CDs. It is unclear whether the weak CDs seen are due to a lack of pigment enantiomer selection or to a disposition of the dipyrnone electric transition dipole moments for the (2.2) and (2.3) – as was seen in earlier studies of the influence of CHCl_3 and volatile anesthetics [42, 43].

Concluding Comments

Like the previously reported (2.2) and (2.3)-homorubins [13, 14], the homorubins of the current work were all predicted to be able to engage in conformation-determining intramolecular hydrogen bonding by molecular mechanics computations. These predictions appear to be borne out experimentally from solubility and chromatographic data along with ^1H NMR N-H chemical shifts for the homorubins of this work, although the higher homologs (4.1), (4.2), and (4.3) and the lower homolog (2.1) are less soluble in CHCl_3 than the (2.2), (2.3), and (3.2). The decreased solubility of the lower and higher homologs suggests an increased polarity that would correlate with less effective intramolecular hydrogen bonding.

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) 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 CDCl_3 unless otherwise indicated. Chemical shifts were reported in ppm referenced to the residual CHCl_3 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 Thomas-Hoover 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. For final purification, radial chromatography was carried out on Merck silica gel PF_{254} 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*-octyl amine acetate in 5% aq. methanol (pH 7.7, 37 $^\circ\text{C}$). 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 6.0 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 Muller and Falk's "Ball and Stick" program (Cherwell Scientific, Oxford, U.K.) for the Macintosh (http://www.orc.uni-linz.ac.at/mueller/ball_and_stick.shtml). All solvents were reagent grade, from Fisher-Acros.

Some synthetic precursors were prepared as in previous work: 3-pyrrolin-2-one (**17**) [29], ethyl 3-[2-(ethoxycarbonyl)ethyl]-4,5-dimethyl-1*H*-pyrrole-2-carboxylate (**19**) [18], ethyl 5-carboxyethyl-2,4-dimethyl-1*H*-pyrrole-3-acetate (**11-1**) [23], and the corresponding 3-propanoate (**11-2**) and 3-butanoate (**11-3**) [23].

Synthesis of (2.1)-homorubin (2-1)

2,2'-(1,2-Ethanediy)bis[5-[(3-ethyl-1,5-dihydro-4-methyl-5-oxo-2*H*-pyrrol-2-ylidene)methyl]-4-methyl-(*Z*)-1*H*-pyrrole-3-acetic acid] (2.1-homorubin) (2-1, $\text{C}_{32}\text{H}_{38}\text{N}_4\text{O}_6$)—Homorubin dimethyl ester **2-1e** (100 mg, 0.166 mmol) was suspended in 5 cm^3 N_2 -purged CH_3OH and 20 cm^3 THF and heated to reflux. Nitrogen-purged NaOH (2 cm^3 , 1 M) was then added, and reflux was continued 4 h. The solution was cooled to 5 $^\circ\text{C}$ and acidified with 10% nitric acid until a precipitate formed. The precipitate was filtered and dried (in vacuo) then redissolved in a minimal amount of CH_2Cl_2 : CH_3OH (75:25 v/v) and

passed through a short column of silica gel using CH₂Cl₂-CH₃OH (95:5 v/v) as eluent. All of the first yellow band was collected giving **2-1**. Yield: 50 mg (0.087 mmol, 52%); m.p.: 270 °C (dec); IR (KBr): $\bar{\nu}$ = 3369, 2970, 1664, 1637, 1274, 1171 cm⁻¹; NMR data in Table 3; UV-Vis data in Table 8.

2,2'-(1,4-Ethanediy)bis[5-[(3-ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-(Z)-1H-pyrrole-3-acetic acid] dimethyl ester [(2.1)-homorubin dimethyl ester] (2-1e, C₃₄H₄₂N₄O₆)—To a 25 cm³ round-bottom flask equipped with a magnetic stirrer and a heating mantle was added 160 mg bis-pyrrolethene tetra-acid **20** (0.408 mmol), 200 mg 5-bromomethylene-3-pyrrolin-2-one (**12**, 0.98 mmol), 15 cm³ CH₃OH, and 8 drops H₂O. The flask was equipped with a condenser and heated under reflux for 8 h. The flask was then cooled and placed in the freezer overnight. The resulting precipitate was collected (vacuum filtration) and washed with cold ethyl acetate to give dimethyl ester **2-1e** as a yellow-orange solid. Yield: 126 mg (0.21 mmol, 51%); m.p.: 245 °C (dec); IR (KBr): $\bar{\nu}$ = 3365, 1736, 1655, 1637, 1234, 1163 cm⁻¹; ¹H NMR: δ = 1.04 (3H, t, *J* = 7.2 Hz), 1.22 (3H, s), 2.13 (3H, s), 2.33 (2H, q, *J* = 7.2 Hz), 3.14 (2H, s), 3.50 (2H, s), 3.70 (3H, s), 5.85 (1H, s), 10.36 (1H, bs), 10.95 (1H, bs) ppm; ¹³C NMR: δ = 7.1, 9.5, 14.7, 17.7, 26.4, 30.5, 51.8, 99.5, 112.4, 122.9, 123.5, 123.6, 128.1, 136.5, 146.7, 172.7, 173.6 ppm; UV-Vis (acetonitrile): λ_{max} (ϵ) = 382 (53200), 403 (sh, 44300) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (acetone): λ_{max} (ϵ) = 387 (54800), 413 (sh, 44300) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (benzene): λ_{max} (ϵ) = 392 (55700), 413 (sh, 49400) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (CH₂Cl₂): λ_{max} (ϵ) = 383 (50000), 407 (sh, 42100) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (CHCl₃): λ_{max} (ϵ) = 395 (51200), 408 (sh, 48900) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis ((CH₃)₂SO): λ_{max} (ϵ) = 415 (57100), 400 (sh, 56700) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (CH₃OH): λ_{max} (ϵ) = 402 (55000) nm (mol⁻¹ dm³ cm⁻¹).

2,2'-(1,2-Ethanediy)bis(5-carboxy-4-methyl-1H-pyrrole-3-acetic acid) (20, C₁₈H₂₀N₂O₈)—To a 100 cm³ round bottom flask equipped with a magnetic stirrer, heating mantle, and a condenser was added 495 mg bis-pyrrolethene **20e** (0.82 mmol), 20 cm³ ethanol (95%), and 20 cm³ NaOH (2 M). The mixture was heated at reflux for 3 h before it was cooled to room temperature, and the ethanol was removed (rotovap). Water (10 cm³) was added, and the solution was cooled to 0 °C using an ice bath. Nitric acid (20%) was added until a white precipitate formed; then a few drops more were added, and the mixture was allowed to stir at 0 °C for 15 min more. The precipitate was collected by filtration, washed with cold H₂O, and dried (vacuum desiccator) overnight to afford the desired **20** as a white powder. Yield: 380 mg (0.82 mmoles, 99%); m.p.: 150–152 °C (dec); IR (KBr): $\bar{\nu}$ = 3450, 1712, 1635, 1460, 1355, 1274 cm⁻¹; ¹H NMR ((CD₃)₂SO): δ = 2.12 (6H, s), 2.65 (4H, s), 11.05 (2N-H, bs), 12.01 (4COOH, bs) ppm; ¹³C NMR ((CD₃)₂SO): δ = 10.2, 25.9, 29.3, 114.3, 117.0, 125.7, 134.2, 162.2, 172.9 ppm.

2,2'-(1,2-Ethanediy)bis[5-(ethoxycarbonyl)-4-methyl-1H-pyrrole-3-acetic acid] diethyl ester (20e, C₂₆H₃₆N₂O₈)—To a 100 cm³ round-bottom flask equipped with a magnetic stirrer was added 100 mg bis-pyrrolethene **21** (0.20 mmol), 100 cm³ ethyl acetate, and 50 mg 5% Pd(C). The flask was equipped with a hydrogen balloon and stirred until the blue fluorescence was no longer visible. More **21** was added, and the balloon was refilled.

This process was repeated as many times as needed (five times for this procedure). The mixture was then filtered through celite, and the ethyl acetate was removed (rotovap). The white solid was recrystallized from ethyl acetate-hexane to give the desired diethyl ester **20e**. Yield: 495 mg (0.99 mmol, 99%); m.p.: 137–138 °C; IR (KBr): ν = 3321, 2985, 1735, 1672, 1437, 1301, 1251, 1020 cm^{-1} ; ^1H NMR: δ = 1.26 (6H, t, J = 7.2 Hz), 1.28 (6H, t, J = 7.2 Hz), 2.23 (6H, s), 2.78 (4H, s), 3.40 (4H, s), 4.17 (4H, q, J = 7.2 Hz), 4.20 (4H, q, J = 7.2 Hz), 9.09 (2N-H, bs) ppm; ^{13}C NMR: δ = 10.1, 14.1, 14.3, 25.8, 29.9, 59.3, 60.9, 113.8, 117.7, 127.0, 132.9, 161.0, 172.7 ppm.

2,2'-(1,2-Ethenediyl)-bis[5-(ethoxycarbonyl)-4-methyl-1H-pyrrole-3-acetic acid] diethyl ester (21, C₂₆H₃₄N₂O₈)—

To a 500 cm^3 round-bottom flask equipped with a magnetic stirrer containing 100 cm^3 dry THF under N_2 was added 8.96 g TiCl_4 (5.3 cm^3 , 47.9 mmol). Upon addition of the TiCl_4 a bright yellow precipitate formed. The mixture was cooled in an ice/salt bath to -5°C . Zinc dust (6.2 g) was added to the mixture over 5 min. The mixture was allowed to warm to room temperature, then heated under reflux for 1 h. The mixture went from yellow to green to dark violet to black. A solution of 2.51 g formylpyrrole **22** (9.6 mmol) in 50 cm^3 dry THF was added to the black mixture. The flask was equipped with a condenser and a heating mantle and heated under reflux for 2 h. The mixture was then cooled to 5 °C, and 200 cm^3 NH_4OH (50%) was slowly added to the mixture. The mixture was poured into a separatory funnel containing 200 cm^3 CH_2Cl_2 and shaken. The organic layer was filtered using aspirator vacuum, and more CH_2Cl_2 was added to the separatory funnel, shaken, and again filtered. This process was repeated until no more yellow solution was extracted. The organic layer was washed with H_2O ($2 \times 100 \text{ cm}^3$) and brine ($1 \times 100 \text{ cm}^3$), dried over anhyd. Na_2SO_4 , and the CH_2Cl_2 was removed (rotovap) to afford a thick yellow paste. Ethanol (95%) was added to thin the paste, and the mixture was filtered and dried (vacuum dessicator) resulting in the yellow **21**. Yield: 1.10 g (2.19 mmol, 46%); m.p.: 206–207 °C; IR (KBr): ν = 3324, 2984, 1694, 1448, 1262, 1161, 958, 772, 665, 631 cm^{-1} ; ^1H NMR: δ = 1.27 (6H, t, J = 7.2 Hz), 1.36 (6H, t, J = 7.2 Hz), 2.25 (6H, s), 3.52 (4H, s), 4.11 (4H, q, J = 7.2 Hz), 4.30 (4H, q, J = 7.2 Hz), 6.74 (2H, s), 9.17 (2N-H, bs) ppm; ^{13}C NMR ($(\text{CD}_3)_2\text{SO}$): δ = 10.2, 13.9, 14.3, 29.1, 59.2, 59.9, 115.0, 117.5, 119.2, 126.7, 132.6, 160.8, 170.8 ppm; UV-Vis (CH_2Cl_2): λ_{max} (ϵ) = 380 (39800) nm ($\text{mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$); UV-Vis (CH_3OH): λ_{max} (ϵ) = 378 (47400), 399 (35600), 361 (sh, 36600), 399 (36000) nm ($\text{mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$); UV-Vis ($(\text{CH}_3)_2\text{SO}$): λ_{max} (ϵ) = 382 (48900), 405 (43200), 367 (sh, 32700) nm ($\text{mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$).

5-(Ethoxycarbonyl)-2-formyl-3-methyl-1H-pyrrole-4-acetic acid ethyl ester (22, C₁₃H₁₇NO₅)—

To a 1 dm^3 round-bottom flask equipped with a magnetic stirrer was added pyrrole 5.0 g **11-1** (20 mmol) and 300 cm^3 acetic acid. After the pyrrole had fully dissolved, 250 cm^3 H_2O and 100 cm^3 THF were added and the solution was cooled to 0 °C. Ceric ammonium nitrate (CAN, 43.5 g, 80 mmol) was added in portions to the solution and allowed to stir 1.5 h. The solution was poured into a 2 dm^3 separatory funnel containing 300 cm^3 CH_2Cl_2 after which 1 dm^3 H_2O was added. The organic layer was washed several times with water to remove as much acetic acid as possible, then washed with 10% aq. NaHCO_3 , followed by brine. The organic layer was dried over anhyd. Na_2SO_4 and removed (rotovap). The remaining oil was passed through a short column of silica gel using CH_2Cl_2 as the

eluent, collected, and evaporated (rotovap). The residue was recrystallized (CH_2Cl_2 -hexane) to give light yellow crystals of **22**. Yield: 3.5 g (13 mmol, 66%); m.p.: 76–77 °C; IR (KBr): $\nu = 3268, 2980, 1736, 1692, 1560, 1484, 1262, 1098, 785 \text{ cm}^{-1}$; ^1H NMR: $\delta = 1.25$ (3H, t, $J = 7.1$ Hz), 1.37 (3H, t, $J = 7.1$ Hz), 2.31 (3H, s), 3.74 (2H, s), 4.15 (2H, q, $J = 7.1$ Hz), 4.35 (2H, q, $J = 7.1$ Hz), 9.61 (1H, bs), 9.78 (N-H, s) ppm; ^{13}C NMR: $\delta = 9.8, 14.1, 14.3, 29.6, 61.0, 61.3, 124.9, 125.6, 127.4, 130.3, 160.8, 170.3, 179.5$ ppm.

Synthesis of (3,2)-homorubin (3-2)

2,2'-(1,3-Propanediyl)bis[5-[(3-ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-(Z)-1H-pyrrole-3-propionic acid] (3-2, $\text{C}_{35}\text{H}_{44}\text{N}_4\text{O}_6$)—

To a 50 cm³ round-bottom flask was added 10 cm³ THF (N_2 -purged) followed by 25 mg dimethyl ester **3-2e** (0.037 mmol), and the mixture was brought to reflux under N_2 . A solution of 3 cm³ CH_3OH and 1 cm³ NaOH (2 M) was added and the mixture was heated at reflux for 4 h under a N_2 atmosphere. After the solution was cooled to room temperature, 5 cm³ H_2O was added and the organic solvents were removed (rotovap). The solution was then cooled to 5 °C, acidified with nitric acid (2 M), and the solid was separated by centrifugation and washed with cold water. The yellow solid was dried under high vacuum to give the desired **3-2**. Yield: 18 mg (0.027 mmol, 74%); IR (KBr): $\nu = 3356, 2967, 2933, 1667, 1634, 1436, 1361, 1174, 980, 677 \text{ cm}^{-1}$; ^1H NMR data in Table 4; UV-Vis data in Table 9.

2,2'-(1,3-Propanediyl)bis[5-[(3-ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-(Z)-1H-pyrrole-3-propionic acid] dimethyl ester (3-2e, $\text{C}_{37}\text{H}_{48}\text{N}_4\text{O}_6$)—

To a 50 cm³ round-bottom flask was added 180 mg **13** (0.279 mmol) and 10 cm³ THF. After all the **13** had dissolved, 10 cm³ CH_3OH was added followed by the addition of 70 mg NaBH_4 . The solution first turned green, then yellow, indicating that the reduction of the azafulvene moiety was complete. To the yellow solution was added 0.5 cm³ acetic acid in order to destroy any unreacted NaBH_4 ; then, 10 cm³ H_2O was added, and a yellow precipitate formed. The organic solvents were removed (rotovap), and the solid residue was taken up in 30 cm³ CH_2Cl_2 . After washing the residue with H_2O (3×30 cm³) and brine (1×30 cm³), then drying it over anhyd. Na_2SO_4 , the solvent was removed (rotovap). The resulting yellow solid was dissolved in CH_2Cl_2 and passed through a short column of silica gel. The isolated, chromatographed yellow solid was determined to be the 1,3-bis propene dipyrinone on the basis of the observation of four N-H resonances, a multiplet near 7.2 ppm and a doublet at 6.45 ppm, which are assigned to the two vinyl proton resonances in the ^1H NMR spectrum. All of the aliphatic ^{13}C resonances were doubled. A portion of the solid was taken directly to the next step.

To a 50 cm³ round-bottomed flask was added 10 cm³ THF and 10 cm³ CH_3OH followed by 10 mg platinum oxide. The flask was then placed on a hydrogenation apparatus and charged with hydrogen. After 30 min, 50 mg of the reduced product from above (0.079 mmol) was dissolved in 5 cm³ THF and added to the pre-hydrogenated platinum oxide and again placed in the hydrogenation apparatus. The mixture was stirred for 24 h, after which the catalyst was removed by filtration and the solvent was removed to give a yellow residue. The residue was dissolved in CH_2Cl_2 and eluted through a short column of silica gel using

CH₂Cl₂:CH₃OH (98:2 v/v), collecting the first yellow band. Hexane (5 cm³) was added to the eluted yellow solution, and the solvent was evaporated (rotovap) until a precipitate formed. The flask was placed in the freezer for 2 h, then filtered to obtain the desired yellow dimethyl ester **3-2e**. Yield: 20 mg (0.031 mmol, 40%); m.p.: 206–207 °C; IR (KBr): $\bar{\nu}$ = 3366, 1255, 1715, 1640, 1636, 1169, 669 cm⁻¹; ¹H NMR: δ = 1.04 (6H, t, *J* = 7.3 Hz), 1.34 (6H, s), 2.10 (6H, s), 2.33 (6H, m), 2.79 (8H, m), 3.73 (6H, s), 5.83 (2H, s), 10.35 (N-H, bs), 11.02 (N-H, bs) ppm; ¹³C NMR: δ = 7.7, 9.5, 14.8, 17.3, 19.9, 25.3, 30.2, 35.6, 51.6, 99.8, 118.7, 122.9, 123.0, 123.4, 127.4, 135.1, 146.8, 173.6, 173.7 ppm; UV-Vis (acetonitrile): λ_{max} (ϵ) = 377 (83300) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (acetone): λ_{max} (ϵ) = 378 (83700) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (benzene): λ_{max} (ϵ) = 382 (85800), 431 (sh, 18300) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (CH₂Cl₂): λ_{max} (ϵ) = 379 (86800), 430 (sh, 17500) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (CHCl₃): λ_{max} (ϵ) = 380 (88600), 430 (sh, 18700) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis ((CH₃)₂SO): λ_{max} (ϵ) = 400 (65900), 419 (sh, 64500) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (CH₃OH): λ_{max} (ϵ) = 413 (65500) nm (mol⁻¹ dm³ cm⁻¹).

5-[(3-Ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-2-[3-[5-[(3-ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-3-[2-(methoxycarbonyl)ethyl]-4-methyl-2H-pyrrol-2-ylidene]-1-propenyl]-4-methyl-1H-pyrrole-3-propionic acid methyl ester (13, C₃₇H₄₄N₄O₆)—To a 100 cm³ round-bottom flask equipped with a magnetic stirred was added 10 cm³ CH₃OH, 20 cm³ CH₂Cl₂, 150 mg propenal dipyrinone **15** (0.42 mmol), and 127 mg dipyrinone **14** (0.42 mmol). Hydrobromic acid in acetic acid (30%, 2 cm³) was slowly added, and the solution was stirred for 30 min. Upon addition of the acid the solution turned red, then dark green. Ammonium hydroxide (~2 cm³) was added to neutralize the solution as it changed color from green to blue. The solution was then poured into a separatory funnel containing 40 cm³ CH₂Cl₂ and the mixture was washed with H₂O (2×50 cm³) and brine (1×50 cm³) then dried over anhyd. Na₂SO₄. The solvent was removed (rotovap) and the residue was redissolved in CH₂Cl₂, and passed through a short column of silica gel using CH₂Cl₂:CH₃OH (98:2 v/v) as eluent while collecting all of the blue compound. Hexane (10 cm³) was added to the blue solution, and the solvent volume was decreased by evaporation until a precipitate formed in the flask. The mixture was placed in the freezer for 2 h, and the precipitate formed was collected by filtration to afford the desired blue **13**. Yield: 230 mg (0.36 mmol, 86%); m.p.: 208–209 °C; IR (KBr): $\bar{\nu}$ = 3424, 2969, 1734, 1699, 1654, 1560, 1280, 1167, 1090, 858, 669 cm⁻¹; ¹H NMR: δ = 0.97 (6H, bs), 1.29 (3H, bs), 1.48 (3H, bs), 2.01 (6H, s), 2.25 (4H, bd), 2.50 (4H, bt, *J* = 7.3 Hz), 2.87 (4H, bt, *J* = 7.3 Hz), 3.70 (6H, s), 5.59 (2H, bs), 6.79 (2H, d, *J* = 13.7 Hz), 8.89 (1H, t, *J* = 13.7 Hz), 10.36 (N-H, bs), 10.84 (N-H, bs), 12.07 (N-H, bs) ppm; UV-Vis (acetonitrile): λ_{max} (ϵ) = 399 (59100) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (acetone): λ_{max} (ϵ) = 403 (62700) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (benzene): λ_{max} (ϵ) = 397 (53300), 413 (sh, 57000) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (CH₂Cl₂): λ_{max} (ϵ) = 398 (54000) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (CHCl₃): λ_{max} (ϵ) = 402 (57000) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis ((CH₃)₂SO): λ_{max} (ϵ) = 412 (58200) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (CH₃OH): λ_{max} (ϵ) = 421 (62000), 401 (sh, 54800) nm (mol⁻¹ dm³ cm⁻¹).

5-[(3-Ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-2-(3-oxo-1(Z)-propenyl)-4-methyl-(Z)-1H-pyrrole-3-propionic acid methyl ester (15,

C₂₀H₂₄N₂O₄)—To a 100 cm³ round bottomed flask equipped with a magnetic stirrer was added 2 cm³ CH₂Cl₂. After cooling to 0 °C, 120 mg dimethylamino-acrolein (1.21 mmol) was added followed by the slow addition of 185 mg phosphorous oxychloride (1.21 mmol) in 2 cm³ CH₂Cl₂. The solution was allowed to warm to room temperature, stirred for 30 min, then cooled to 0 °C. A solution of 300 mg dipyrinone **14** (1.01 mmol) in 5 cm³ CH₂Cl₂ was added slowly, immediately giving a red solution. The solution was stirred for 30 min before a solution of sodium acetate (1 g in 50 cm³ H₂O) was added; then, stirring was continued for 8 h. The organic layer was washed with H₂O (2×50 cm³), dried over anhyd. Na₂SO₄, and removed (rotovap), to yield a red-yellow solid. The solid was flash chromatographed using CH₂Cl₂:CH₃OH (98:2 v/v) as eluent. A yellow band was collected, and the solvent removed (rotovap). Recrystallization of the residue from CH₂Cl₂-hexane gave the desired **15**. Yield: 81 mg (0.24 mmol, 70% yield); m.p.: 233–234 °C; IR (KBr): ν = 3350, 2933, 1733, 1669, 1648, 1615, 1272, 1167, 1119 cm⁻¹; ¹H NMR: δ = 1.18 (3H, t, *J* = 7.3 Hz), 1.91 (3H, s), 2.16 (3H, s, 3H), 2.52 (4H, m), 2.94 (2H, t, *J* = 7.3 Hz), 3.69 (3H, s), 6.09 (1H, s), 6.95 (1H, dd, *J* = 7.8 Hz, 15.6 Hz), 7.42 (1H, d, *J* = 15.6 Hz), 9.60 (1H, d, 7.8 Hz), 10.40 (N-H, bs), 11.21 (N-H, bs) ppm; ¹³C NMR: δ = 8.7, 9.3, 14.8, 17.9, 19.8, 34.9, 51.8, 98.7, 123.7, 125.7, 126.1, 129.6, 129.8, 130.1, 123.1, 138.5, 148.7, 173.0, 175.1, 193.4 ppm; UV-Vis (acetonitrile): λ_{max} (ϵ) = 433 (26100) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (acetone): λ_{max} (ϵ) = 435 (34300) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (benzene): λ_{max} (ϵ) = 470 (21800) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (CH₂Cl₂): λ_{max} (ϵ) = 440 (21100) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis ((CH₃)₂SO): λ_{max} (ϵ) = 451 (32400) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (CH₃OH): λ_{max} (ϵ) = 445 (33800) nm (mol⁻¹ dm³ cm⁻¹).

5-[(3-Ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-(Z)-1H-pyrrole-3-propionic acid methyl ester (14, C₁₇H₂₁N₂O₃)—To a 100 cm³ round-bottom flask equipped with a magnetic stirrer and heating mantle was added 1.15 g formylpyrrole **18** (7.2 mmol), 1.80 g 3-pyrrolin-2-one **17** [29] (6.5 mmol), and 40 cm³ CH₃OH. Aqueous KOH (100 cm³, 4 M) was added, and the flask was fitted with a condenser. The solution was heated at reflux under N₂ in the dark. After 16 h, the solution was cooled to room temperature and washed with CH₂Cl₂. The aqueous layer was then cooled in an ice bath and acidified with 20% HCl to form a yellow green precipitate of **16**. The precipitate was centrifuged and washed with water in the centrifuge tube. The precipitate was filtered (suction filtration) using a minimal amount of water. Suction was continued for ~3 h. The green solid (**16**) was suspended in 100 cm³ CH₃OH and brought to reflux. Sulfuric acid (4 cm³, 2 M) was added and reflux was continued until most all of the solid was in solution (~2 h). The mixture was cooled to room temperature and neutralized with 5% aq. NaHCO₃. The CH₃OH was then removed (rotovap), and the residue was dissolved in 100 cm³ CH₂Cl₂. The organic layer was washed with H₂O (2×100 cm³) and brine (1×100 cm³), dried over anhyd. Na₂SO₄, and the solvent was removed (rotovap). The residue was redissolved in CH₂Cl₂, passed through a short column of silica gel, and the yellow fraction collected while eluted by CH₂Cl₂:CH₃OH (98:2 v/v). After removal of the solvent, the residue was recrystallized from CH₃OH giving the desired **14**. Yield: 1.16 g (3.84 mmol, 59%); m.p.: 174–175 °C (Ref. [34] 175–176 °C); IR (KBr): ν = 3759, 2916, 1716, 1661, 1636, 1173 cm⁻¹; ¹H NMR: δ = 1.18 (3H, t, *J* = 7.3 Hz), 1.95 (3H, s), 2.15 (3H, s), 2.55 (2H, q, *J* = 7.3 Hz), 2.58 (2H, d, 7.3 Hz), 2.79 (2H, d, *J* = 7.3 Hz), 3.69 (3H, s), 6.15

(1H, s), 6.85 (1H, s), 10.53 (N-H, bs), 11.08 (N-H, bs) ppm; ^{13}C NMR: δ = 8.1, 9.4, 14.9, 18.0, 20.7, 34.8, 51.6, 101.1, 120.7, 122.6, 123.3, 123.4, 124.4, 128.5, 148.5, 173.7, 174.3 ppm.

2-(Ethoxycarbonyl)-5-formyl-4-methyl-1H-pyrrole-3-propionic acid ethyl ester (18, C₁₄H₁₅NO₅)—To a 1000 cm³ round-bottom flask equipped with a magnetic stirrer was added 5.0 g pyrrole **19** [18] (18.7 mmol), 100 cm³ THF, 300 cm³ acetic acid, and 250 cm³ H₂O. The mixture was stirred until all of the pyrrole had dissolved then cooled to 5 °C. Ceric ammonium nitrate (CAN, 41.0 g, 74.8 mmol) was added, and the mixture was allowed to stir for 1 h. Water (500 cm³) was then added, and the solution was extracted with CH₂Cl₂ (2×175 cm³), washed with H₂O (2×150 cm³), 5% aq. NaHCO₃ (1×200 cm³), H₂O (1×150 cm³), and brine (1×150 cm³). The organic layer was dried (anhyd. Na₂SO₄), filtered, and evaporated (rotovap). The oily residue was crystallized from CH₂Cl₂-hexane to give the desired α -aldehyde **18**. Yield: 4.6 g (16.4 mmol, 91%); m.p.: 83–84 °C (Ref. [30] 84–86 °C); IR (KBr): ν = 3142, 2982, 1731, 1704, 1557, 1274, 1251, 1129, 1024, 780, 669 cm⁻¹; ^1H NMR: δ = 1.24 (3H, t, J = 7.3 Hz), 1.38 (3H, t, J = 7.3 Hz), 2.33 (1H, s), 2.53 (2H, t, J = 7.3 Hz), 3.03 (2H, t, J = 7.3 Hz), 4.11 (2H, q, J = 7.3 Hz), 4.35 (2H, q, J = 7.3 Hz), 9.57 (N-H, bs), 9.77 (1H, s) ppm; ^{13}C NMR: δ = 8.3, 14.2, 14.2, 19.8, 35.5, 60.3, 61.1, 124.2, 125.8, 129.7, 131.4, 160.3, 172.8, 179.1 ppm.

Synthesis of (4.1)-homorubin (4-1)

2,2'-(1,4-Butanediyl)bis[5-[(3-ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-(Z)-1H-pyrrole-3-acetic acid] [(4.1)-homorubin] (4-1, C₃₄H₄₂N₂O₆)—Homorubin dimethyl ester **4-1e** (50 mg, 0.079 mmol) was suspended in 20 cm³ N₂-purged CH₃OH and 5 cm³ THF and heated to reflux. Nitrogen-purged aq. NaOH (2 cm³, 1 M) was added, and reflux was continued for 4 h under a blanket of N₂ before it was cooled to 5 °C and acidified with nitric acid (10%) to form a precipitate. The precipitate was collected by filtration and dried in vacuo. Then it was redissolved in a minimal amount of dimethyl formamide and heated to ~90 °C. Upon cooling to room temperature, the precipitate that had formed was collected by filtration, washed with ethanol, and dried to afford the desired **4-1**. Yield: 20 mg (0.033 mmol, 43%); m.p.: 240 °C (dec); IR (KBr): ν = 3356, 2968, 1667, 1635, 1456, 1270, 1171, 980, 668 cm⁻¹; NMR data in Table 3; UV-Vis data in Table 8.

2,2'-(1,4-Butanediyl)bis[5-[(3-ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-(Z)-1H-pyrrole-3-acetic acid] dimethyl ester [(4.1)-homorubin dimethyl ester] (4-1e, C₃₆H₄₆N₄O₆)—To a 25 cm³ round-bottom flask equipped with a magnetic stirrer and heating mantle was added 117 mg bis-pyrrolbutane tetra-acid **5-1** (0.279 mmol), 120 mg 5-bromomethylene-3-pyrrolin-2-one (**12**, 0.558 mmol), and 7 cm³ CH₃OH. The mixture was heated at reflux under a blanket of N₂ (after ~30 minutes it becomes a solution). After 3 h, a precipitate began to form. Heat was removed after 12 h, and the mixture was placed in the freezer overnight. The precipitate was collected by filtration, then suspended in 5 cm³ dimethyl formamide and heated until all the solid had dissolved (~110 °C). As the solution cooled to room temperature a precipitate formed. The precipitate was isolated by filtration and washed with ethanol (95%) to yield dimethyl ester

4-1e as a yellow-green solid. Yield: 71 mg (0.117 mmol, 42%); m.p.: 250 °C (dec); IR (KBr): ν = 3336, 2937, 1733, 1667, 1634, 1457, 1436, 1269, 1111, 979, 678 cm⁻¹; ¹H NMR ((CD₃)₂SO): δ = 1.02 (6H, t, J = 7.3 Hz), 1.51 (3H, brs), 1.78 (6H, s), 2.00 (6H, s), 2.45–2.51 (8H, brm), 3.35 (4H, s), 3.51 (6H, s), 5.94 (2H, s), 9.83 (2N-H, brs), 10.31 (2N-H, brs) ppm; ¹³C NMR ((CD₃)₂SO): δ = 8.1, 9.3, 14.8, 17.4, 25.4, 29.6, 29.7, 51.4, 97.5, 112.9, 122.0, 122.7, 122.9, 127.7, 134.9, 147.3, 171.9, 172.0 ppm; UV-Vis (acetonitrile): λ_{max} (ϵ) = 399 (59100) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (acetone): λ_{max} (ϵ) = 403 (62200) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (benzene): λ_{max} (ϵ) = 397 (53300), 413 (sh, 57000) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (CH₂Cl₂): λ_{max} (ϵ) = 398 (54000) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (CHCl₃): λ_{max} (ϵ) = 402 (56800) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis ((CH₃)₂SO): λ_{max} (ϵ) = 412 (58200) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (CH₃OH): λ_{max} (ϵ) = 421 (62000) nm (mol⁻¹ dm³ cm⁻¹).

2,2'-(1,4-Butanediyl)bis(5-carboxy-4-methyl-1H-pyrrole-3-acetic acid) (5-1, C₂₀H₂₄N₂O₈)—Bis-pyrrolbutane **5-1e** (210 mg, 0.395 mmol) was dissolved in 10 cm³ warm CH₃OH. Aqueous NaOH (10 cm³, 2 M) was added, and the solution was heated under reflux for 6 h. The CH₃OH was then removed (rotovap), and the aqueous layer was cooled to 5 °C, then acidified with nitric acid (20%) until a white precipitate fell out of solution. The solid was collected by filtration and dried in vacuo to give the desired **5-1**. Yield: 120 mg (0.29 mmol, 72%). The tetra-acid was characterized as follows and used directly in the next step. M.p.: 128 °C (dec); ¹H NMR ((CD₃)₂SO): δ = 1.43 (4H, brs), 2.10 (6H, s), 2.44 (4H, brs), 3.20 (4H, s), 10.92 (2N-H, brs), 11.87 (2COOH, brs) ppm; ¹³C NMR ((CD₃)₂SO): δ = 10.3, 24.8, 29.0, 29.6, 113.9, 116.7, 125.9, 135.2, 162.2, 172.8 ppm.

2,2'-(1,4-Butanediyl)bis[(5-ethoxycarbonyl)-4-methyl-1H-pyrrole-3-acetic acid] diethyl ester (5-1e, C₂₈H₄₀N₂O₈)—Bis-pyrrol butadiyne **6-1e** (200 mg, 0.38 mmol) was partially dissolved in 50 cm³ ethyl acetate, followed by the addition of 50 mg palladium on carbon (5%). The flask was equipped with a hydrogen balloon, and the mixture was stirred until it became a dark black mixture (~1 h). The mixture was then filtered through celite, and the solvent was removed to give a white solid. The latter was recrystallized from CH₂Cl₂-hexane to afford white crystals of **5-1e**. Yield: 195 mg (0.37 mmol, 97 %); m.p.: 140–142 °C; IR (KBr): ν = 3305, 2981, 2938, 1732, 1668, 1450, 1431, 1281, 1084, 922 cm⁻¹; ¹H NMR: δ = 1.22 (6H, t, J = 7.3 Hz), 1.33 (6H, t, J = 7.3 Hz), 1.61 (4H, t, J = 7.2 Hz), 2.27 (6H, s), 2.57 (4H, t, J = 7.2 Hz), 3.36 (4H, s), 4.10 (4H, q, J = 7.3 Hz), 4.28 (4H, q, J = 7.3 Hz), 8.96 (2N-H, bs) ppm; ¹³C NMR: δ = 10.6, 14.2, 14.5, 25.6, 28.8, 30.2, 59.7, 60.7, 114.4, 117.6, 127.4, 134.7, 167.7, 171.8 ppm.

2,2'-(1,4-Butadiynediyl)bis[(5-ethoxycarbonyl)-4-methyl-1H-pyrrole-3-acetic acid] diethyl ester (6-1e, C₂₈H₃₂N₂O₈)—A mixture of 500 mg ethynyl pyrrole **7-1** (1.90 mmol) and 214 mg chloroacetone in 25 cm³ dry benzene was added to a solution of 47 mg tetrakis(triphenylphosphine)palladium(0), 24 mg copper(I) iodide, and 380 mg triethylamine in 25 cm³ dry benzene. After stirring the reaction mixture at room temperature for 16 h the solvent was removed (rotovap) and the residue was taken into CH₂Cl₂. The solution was passed through a short column of silica gel, eluting first with CH₂Cl₂ then CH₂Cl₂:CH₃OH (97.25:0.75 v/v). All of the yellow solution was collected, and the solvent removed (roto-vap). The yellow residue was recrystallized from CH₂Cl₂-hexane to give

light yellow crystals of the desired **6-1e**. Yield: 449 mg (0.86 mmol, 91%); m.p.: 212–213 °C; IR (KBr): $\bar{\nu}$ = 3254, 2981, 2931, 2142, 1733, 1633, 1468, 1268, 1177, 1032, 750 cm^{-1} ; ^1H NMR: δ = 1.27 (6H, t, J = 7.3 Hz), 1.36 (6H, t, J = 7.3 Hz), 2.26 (6H, s), 3.55 (4H, s), 4.17 (4H, q, J = 7.3 Hz), 4.37 (4H, q, J = 7.3 Hz), 9.22 (2N-H, bs) ppm; ^{13}C NMR: δ = 10.0, 13.8, 14.0, 30.8, 59.6, 60.2, 74.8, 78.2, 113.7, 120.9, 125.1, 125.7, 159.8, 170.0 ppm; UV-Vis (CH_2Cl_2): λ_{max} (ϵ) = 329 (40800) nm ($\text{mol}^{-1} \text{dm}^3 \text{cm}^{-1}$); UV-Vis (CH_3OH): λ_{max} (ϵ) = 328 (44700) nm ($\text{mol}^{-1} \text{dm}^3 \text{cm}^{-1}$); UV-Vis ($(\text{CH}_3)_2\text{SO}$): λ_{max} (ϵ) = 331 (38600) nm ($\text{mol}^{-1} \text{dm}^3 \text{cm}^{-1}$).

5-(Ethoxycarbonyl)-2-ethynyl-4-methyl-1H-pyrrole-3-acetic acid ethyl ester

(7-1, C₁₄H₁₇NO₄)—The TMS-acetylene pyrrole **8-1** (2.0 g, 5.96 mmol) was dissolved in 30 cm^3 dry THF. Anhydrous ethanol (50 cm^3) and 1.30 g K_2CO_3 were then added, and the mixture was stirred for 4 h at room temperature. The mixture was poured into 40 cm^3 H_2O and the ethanol and THF were removed (rotovap). The aqueous mixture was extracted with CH_2Cl_2 (3×25 cm^3). The combined organic layers were washed with H_2O (2×75 cm^3), brine (1×100 cm^3), and dried over anhyd. Na_2SO_4 . The solvent was removed (rotovap), and the crude residue was taken into a small amount of CH_2Cl_2 and filtered through a short column of silica gel, from which the entire first yellow band to elute was collected. After removing the solvent, the yellow-brown solid was recrystallized from hexane to afford the desired ethyl ester **7-1**. Yield: 1.04 g (3.97 mmol, 66%); m.p.: 110–111 °C; IR (KBr): $\bar{\nu}$ = 3265, 2993, 2091, 1731, 1683, 1458, 1270, 1171, 1043, 1022 cm^{-1} ; ^1H NMR: δ = 1.24 (3H, t, J = 7.3 Hz), 1.34 (3H, t, J = 7.3 Hz), 2.25 (3H, s), 3.34 (1H, s), 3.52 (2H, s), 4.14 (2H, q, J = 7.3 Hz), 4.32 (2H, q, J = 7.3 Hz), 9.29 (N-H, bs) ppm; ^{13}C NMR: δ = 10.4, 14.5, 14.4, 30.7, 60.3, 60.7, 74.3, 82.7, 114.8, 120.2, 123.5, 126.5, 160.8, 170.8 ppm.

5-(Ethoxycarbonyl)-4-methyl-2-[(trimethylsilyl)ethynyl]-1H-pyrrole-3-acetic acid ethyl ester (8-1, C₁₇H₂₅NO₄Si)

—To a solution of 3 g iodo-pyrrole **9-1** (8.22 mmol) in 85 cm^3 diethylamine were added under N_2 0.90 g (trimethylsilyl)acetylene (9.20 mmol), 101 mg bis(triphenylphosphine)palladium(II) chloride, and 54 mg copper(I) iodide. The mixture was stirred at 50 °C for 1.5 h. The solvent was removed (rotovap) at water aspirator vacuum, the residue was taken into CH_2Cl_2 , and passed through a short column of silica gel using CH_2Cl_2 as the eluent. All of the first yellow band to elute was collected, the solvent was removed (rotovap), and the residue was recrystallized from hexane to afford the desired ethyl ester **8-1**. Yield: 2.47 g (7.40 mmol, 90%) m.p.: 85–86 °C; IR (KBr): $\bar{\nu}$ = 3276, 2664, 2149, 1745, 1671, 1475, 1275, 1160, 1027, 845 cm^{-1} ; ^1H NMR: δ = 231 (9H, s), 1.25 (3H, t, J = 7.3 Hz), 1.35 (3H, t, J = 7.3 Hz), 2.25 (3H, s), 3.51 (2H, s), 4.14 (2H, q, J = 7.3 Hz), 4.30 (2H, q, J = 7.3 Hz), 8.98 (N-H, bs) ppm; ^{13}C NMR: δ = −0.34, 10.3, 14.0, 14.3, 30.7, 60.1, 60.5, 94.7, 100.6, 115.7, 119.7, 123.2, 126.5, 160.5, 170.7 ppm.

5-(Ethoxycarbonyl)-2-iodo-4-methyl-1H-pyrrole-3-acetic acid ethyl ester (9-1, C₁₂H₁₆I₂NO₄)

—A solution of 2.10 g NaHCO_3 (25.0 mmol) in 4 cm^3 H_2O was added at 65 °C to a stirred solution of 2.34 g carboxypyrrole **10-1** (8.2 mmol) in 40 cm^3 ethanol. Then a solution of 2.10 g I_2 (8.20 mmol) and 2.74 g KI (16.6 mmol) in 60 cm^3 ethanol and 15 cm^3 H_2O was added drop-wise during 1 h while the temperature was maintained at 60 °C. After stirring at 60 °C for 1 h, the solution was allowed to stir at room temperature for an

additional 2 h. To the light pink solution was added 60 cm³ H₂O containing 1 g Na₂S₂O₂, thus giving a white precipitate. The mixture was placed in the freezer 1 h; then, the solid was collected by filtration and dried to afford ethyl ester **9-1**. Yield: 2.30 g (6.30 mmol, 78%); m.p.: 117–118 °C; IR (KBr): ν = 3275, 2983, 1737, 1681, 1245, 1211, 1132, 1063 cm⁻¹; ¹H NMR: δ = 1.26 (3H, t, *J* = 7.3 Hz), 1.36 (3H, t, *J* = 7.3 Hz), 2.31 (3H, s), 3.40 (2H, s), 4.15 (2H, q, *J* = 7.3 Hz), 4.33 (2H, q, *J* = 7.3 Hz), 9.18 (N-H, bs) ppm; ¹³C NMR: δ = 11.0, 14.2, 14.5, 32.8, 60.3, 60.8, 74.2, 123.9, 129.3, 127.4, 160.6, 170.7 ppm.

2-Carboxy-5-(ethoxycarbonyl)-4-methyl-1H-pyrrole-3-acetic acid ethyl ester (10-1, C₁₃H₁₇NO₆)—Sulfonyl chloride (7.93 g, 4.75 cm³, 59.4 mmol) was added dropwise to a stirred solution of 5 g pyrrole **11-1** (19.8 mmol) dissolved in 100 cm³ anhyd. ether at 5 °C. Stirring was continued for three days at room temperature; then, the ether was removed. The remaining oil was taken into 50 cm³ dioxane and sodium acetate (5 g in 50 cm³ H₂O) was added, and the solution was stirred overnight. The solution was extracted with ether (3×50 cm³), after which the ether was extracted with sat. aq. Na₂CO₃. The aq. layer was then cooled in an ice bath and acidified with nitric acid (20%) as a precipitate formed. The precipitate was collected by filtration, washed with water, and dried in vacuo to afford ethyl ester **10-1**. Yield: 3.5 g (12.4 mmol, 63%); m.p.: 187–188 °C; IR (KBr): ν = 3291, 2983, 1737, 1714, 1472, 1213, 1201, 1020, 928 cm⁻¹; ¹H NMR ((CD₃)₂SO): δ = 1.05 (3H, t, *J* = 7.3 Hz), 1.29 (3H, t, *J* = 7.3 Hz), 2.15 (3H, s), 3.76 (2H, s), 4.03 (2H, q, *J* = 7.3 Hz), 4.22 (2H, q, *J* = 7.3 Hz), 11.75 (N-H, bs), 12.95 (COOH, bs) ppm; ¹³C NMR ((CD₃)₂SO): δ = 9.9, 14.2, 14.2, 29.9, 59.9, 60.1, 121.5, 122.7, 123.5, 126.6, 160.5, 161.8, 170.8 ppm.

Synthesis of (4.2)-homorubin (4-2)

2,2'-(1,4-Butanediyl)bis[5-[(3-ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-(Z)-1H-pyrrole-3-propanoic acid] [(4.2)-homorubin] (4-2, C₃₆H₄₆N₄O₆)—Homorubin dimethyl ester **4-2e** (30 mg, 0.046 mmol) was suspended in 3 cm³ N₂-purged CH₃OH and 6 cm³ THF and heated to reflux. Nitrogen-purged aq. NaOH (2 cm³, 1 M) was added, and reflux was continued for 4 h under N₂ before the solution was cooled to room temperature. Then 3 cm³ H₂O was added, and the organic solvents were removed (rotovap). The mixture was cooled to 5 °C and acidified with nitric acid (10%) until a precipitate formed. The precipitate was collected by filtration and dried in vacuo; then, it was redissolved in a minimal amount of hot dimethyl formamide (~90 °C) and allowed to cool to room temperature during which time a precipitate formed. The solid was collected by filtration, washed with ethanol (95%), and dried in vacuo to give the desired **4-2**. Yield: 18 mg (0.028 mmol, 62%); m.p.: 290 °C (dec); IR (KBr): ν = 3419, 2934, 1652, 1635, 1270, 1173, 980, 679 cm⁻¹; NMR data in Table 4; UV-Vis data in Table 9.

2,2'-(1,4-Butanediyl)bis[5-[(3-ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-(Z)-1H-pyrrole-3-propanoic acid] dimethyl ester [(4.2)-homorubin dimethyl ester] (4-2e, C₃₈H₅₀N₄O₆)—To a 50 cm³ round-bottom flask equipped with a magnetic stirrer and a heating mantle were added 230 mg bis-pyrrolbutane tetra-acid **5-2** (0.51 mmol), 250 mg 5-bromomethylene-3-pyrroline-2-one (**12**,

1.16 mmol), 15 cm³ CH₃OH, and 5 drops H₂O. The mixture was heated at reflux under a blanket of N₂. After 5 h, a precipitate began to fall out of solution, and after 8 h heating was stopped, and the mixture was placed in the freezer overnight. The precipitate was collected by filtration and dissolved in hot dimethyl formamide (~110 °C), then allowed to cool to room temperature, during which time a precipitate formed. The precipitate was collected by filtration and washed with ethanol (95%) and dried in vacuo to afford a yellow green solid dimethyl ester **4-2e**. Yield: 150 mg (0.228 mmol, 45%); m.p.: 280 °C (dec); IR (KBr): ν = 3356, 2934, 1735, 1668, 1635, 1270, 1173, 980, 669 cm⁻¹; ¹H NMR ((CD₃)₂SO): δ = 1.04 (6H, t, *J* = 7.3 Hz), 1.51 (4H, brm), 1.74 (6H, s), 1.97 (6H, s), 2.31 (4H, t, *J* = 7.2 Hz), 2.45–2.51 (8H, brm), 3.52 (6H, s), 5.88 (2H, s, 2H), 9.79 (2N-H, brs), 10.15 (2N-H, brs) ppm; ¹³C NMR ((CD₃)₂SO): δ = 8.0, 9.1, 14.7, 17.1, 19.4, 25.4, 30.0, 35.0, 51.1, 97.5, 118.2, 122.0, 122.1, 122.8, 127.6, 133.9, 147.2, 171.9, 172.71 ppm; UV-Vis (acetonitrile): λ_{max} (ϵ) = 399 (57400) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (acetone): λ_{max} (ϵ) = 405 (59000) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (benzene): λ_{max} (ϵ) = 401 (55600), 415 (sh, 55100) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (CH₂Cl₂): λ_{max} (ϵ) = 397 (54000) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (CHCl₃): λ_{max} (ϵ) = 402 (54000) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis ((CH₃)₂SO): λ_{max} (ϵ) = 413 (58200) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (CH₃OH): λ_{max} (ϵ) = 412 (58300) nm (mol⁻¹ dm³ cm⁻¹).

2,2'-(1,4-Butanediyl)bis(5-carboxy-4-methyl-1H-pyrrole-3-propanoic acid) (5-2, C₂₂H₂₈N₂O₈)—Bis-pyrrolbutane tetra-ester **5-2e** (315 mg, 0.540 mmol) was dissolved in 10 cm³ warm ethanol. Aqueous NaOH (20 cm³, 2 M) was added, and the solution was heated under reflux for 6 h. The ethanol was removed (rotovap), and the aqueous layer was cooled to 5 °C, then acidified with nitric acid (20%) until a white precipitate fell out of solution. The solid was collected by filtration and dried in vacuo to afford the desired **5-2**. Yield: 240 mg (0.535 mmol, 99 %). It was characterized as follows and carried directly to the next step. M.p.: 172 °C (dec); ¹H NMR ((CD₃)₂SO): δ = 1.60 (2H, brs), 2.13 (3H, s, 3H), 2.25 (2H, brs), 2.4–2.5 (4H, brs), 10.79 (N-H, brs), 12.01 (COOH, 2H) ppm; ¹³C NMR ((CD₃)₂SO): δ = 10.1, 19.2, 24.8, 29.4, 35.2, 116.7, 118.8, 125.1, 134.2, 162.1, 173.7 ppm.

2,2'-(1,4-Butanediyl)bis[5-(ethoxycarbonyl)-4-methyl-1H-pyrrole-3-propanoic acid] diethyl ester (5-2e, C₃₀H₄₄N₂O₈)—Diethyl ester **6-2e** (400 mg, 0.72 mmol) was dissolved in 200 cm³ ethyl acetate, followed by the addition of 50 mg palladium on carbon (5%). The flask was fitted with a hydrogen balloon and the mixture was stirred until fluorescence was no longer observed (~4 h). The mixture was filtered through celite and the solvent was removed (rotovap) to give a quantitative yield of the desired **5-2e**. Yield: 402 mg (0.724 mmol); m.p.: 116–117 °C; IR (KBr): ν = 3302, 2932, 1733, 1662, 1274, 1169, 1095, 1028 cm⁻¹; ¹H NMR: δ = 1.23 (6H, t, *J* = 7.3 Hz), 1.33 (6H, t, *J* = 7.2 Hz), 1.60 (4H, brs), 2.26 (6H, s), 2.39 (4H, t, *J* = 7.3 Hz), 2.54 (4H, brs), 2.69 (4H, t, *J* = 7.3 Hz), 4.10 (4H, q, *J* = 7.3 Hz), 4.28 (4H, q, *J* = 7.3 Hz), 8.71 (2N-H, bs) ppm; ¹³C NMR: δ = 10.5, 14.2, 14.6, 19.6, 25.7, 29.2, 35.4, 59.6, 60.3, 117.5, 20.0, 126.9, 133.6, 161.6, 173.0 ppm.

2,2'-(1,4-Butadiynediyl)bis[5-(ethoxycarbonyl)-4-methyl-1H-pyrrole-3-propanoic acid] diethyl ester (6-2e, C₃₀H₃₆N₂O₈)—A mixture of the 690 mg 5-(ethoxycarbonyl)-2-ethynyl-4-methyl-1H-pyrrole-3-propanoic acid ethyl ester (**7-2**, 2.5

mmol) and 280 mg chloroacetone in 30 cm³ dry benzene was added to a solution of 62 mg tetrakis(triphenylphosphine)palladium(0), 31 mg copper(I) iodide, and 500 mg triethylamine in 30 cm³ dry benzene. After stirring the mixture at room temperature for 16 h, the solvent was removed (rotovap). The residue was taken into CH₂Cl₂, passed through a short column of silica gel first using CH₂Cl₂ as eluent, then CH₂Cl₂:CH₃OH (99:1 v/v). The entire yellow band was collected, and the solvent was removed to give a yellow residue. This was recrystallized from hexane to give the desired **6-2e** as light yellow crystals. Yield: 479 mg (1.75 mmol, 70%); m.p.: 165–166 °C; IR (KBr): ν = 3256, 2977, 2143, 1736, 1671, 1465, 1447, 1264, 1176, 1161, 1094, 776 cm⁻¹; ¹H NMR: δ = 1.26 (6H, t, *J* = 7.3 Hz), 1.36 (6H, t, *J* = 7.3 Hz), 2.29 (6H, s), 2.55 (4H, t, *J* = 7.3 Hz), 2.86 (4H, t, *J* = 7.3 Hz), 4.14 (4H, q, *J* = 7.3 Hz), 4.33 (4H, q, *J* = 7.3 Hz), 9.06 (2N-H, bs) ppm; ¹³C NMR: δ = 10.0, 14.0, 14.3, 20.3, 34.5, 60.2, 60.2, 74.5, 79.2, 114.6, 121.0, 125.9, 131.6, 160.3, 172.4 ppm; UV-Vis (CH₂Cl₂): λ_{max} (ϵ) = 333 (42500) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis (CH₃OH): λ_{max} (ϵ) = 329 (51400) nm (mol⁻¹ dm³ cm⁻¹); UV-Vis ((CH₃)₂SO): λ_{max} (ϵ) = 330 (39600) nm (mol⁻¹ dm³ cm⁻¹).

5-(Ethoxycarbonyl)-2-ethynyl-4-methyl-1H-pyrrole-3-propanoic acid ethyl ester (7-2, C₁₅H₁₉NO₄)—Ethyl ester **8-2** (1.11 g, 3.18 mmol) was dissolved in 15 cm³ dry THF.

Anhydrous ethanol (40 cm³) and 800 mg K₂CO₃ were added, and the mixture was stirred for 4 h at room temperature. The mixture was then poured into 60 cm³ water, and the ethanol and THF were removed (rotovap). The resulting aqueous solution was extracted with CH₂Cl₂ (3×50 cm³), and the organic layer was washed with H₂O (2×75 cm³) and brine (1×100 cm³) and dried over anhyd. Na₂SO₄. The solvent was removed (rotovap) and the crude residue was taken into a small amount of CH₂Cl₂ and filtered through a short column of silica gel of which all of the first yellow band was collected. After removing the solvent (rotovap) the yellow-brown solid was recrystallized from hexane, giving the desired ethyl ester **7-2**. Yield: 690 mg (2.50 mmol, 79%); m.p.: 104–105 °C; IR (KBr): ν = 3272, 2994, 1717, 1671, 1273, 1172, 1089, 1033 cm⁻¹; ¹H NMR: δ = 1.23 (3H, t, *J* = 7.3 Hz), 1.34 (3H, t, *J* = 7.3 Hz), 2.27 (3H, s), 2.52 (3H, t, *J* = 7.3 Hz), 2.82 (2H, t, *J* = 7.3 Hz), 3.35 (1H, s), 4.12 (2H, q, *J* = 7.3 Hz), 4.31 (2H, q, *J* = 7.3 Hz), 9.16 (N-H, bs) ppm; ¹³C NMR: δ = 10.2, 14.1, 14.4, 20.2, 34.6, 60.2, 60.3, 74.7, 82.6, 119.9, 121.8, 125.8, 129.3, 161.8, 172.8 ppm.

5-(Ethoxycarbonyl)-4-methyl-2-[(trimethylsilyl)ethynyl]-1H-pyrrole-3-propanoic acid ethyl ester [32] (8-2, C₁₈H₂₇NO₄Si)—As for the synthesis of **8-1**, using an

ethynylation procedure developed earlier [32], to a solution of 2 g iodopyrrole **9-2** (5.30 mmol) in 60 cm³ diethylamine were added under N₂ 0.78 g (trimethylsilyl)acetylene (7.90 mmol), 65 mg bis(triphenylphosphine)palladium(II) chloride, and 65 mg copper(I) iodide. The reaction and work-up proceeded as for **8-1** to give the desired ethyl ester **8-2**. Yield: 1.40 g (4.0 mmol, 76%); m.p.: 83–84 °C; IR (KBr): ν = 3277, 2982, 2149, 1731, 1674, 1456, 1273, 1162, 1095, 1031, 841 cm⁻¹; ¹H NMR: δ = 0.219 (9H, s), 1.22 (3H, t, *J* = 7.3 Hz), 1.33 (3H, t, *J* = 7.3 Hz), 2.25 (3H, s), 2.52 (3H, t, *J* = 7.3 Hz), 2.80 (2H, t, *J* = 7.3 Hz), 4.12 (2H, q, *J* = 7.3 Hz), 4.29 (2H, q, *J* = 7.3 Hz), 9.15 (N-H, bs) ppm; ¹³C NMR: δ = -0.3, 10.2, 14.2, 14.4, 20.3, 34.5, 60.1, 60.2, 94.4, 100.5, 114.9, 119.6, 125.8, 129.3, 160.8, 172.8 ppm.

5-(Ethoxycarbonyl)-2-iodo-4-methyl-1H-pyrrole-3-propanoic acid ethyl ester (9-2, C₁₃H₁₈INO₄)—As for **9-1**, a solution of 2.7 g NaHCO₃ (24.6 mmol) in 6 cm³ H₂O was added at 65 °C to a stirred solution of 3.0 g carboxypyrrole **10-2** (10.2 mmol) in 50 cm³ CH₃OH. A solution of 2.6 g I₂ (10.2 mmol) and 3.4 g KI (20.4 mmol) in 60 cm³ CH₃OH and 15 cm³ H₂O was added drop-wise during 1 h while the temperature was maintained at 60 °C. The reaction and work-up proceeded as for **8-1** to afford ethyl ester **9-2**. Yield: 2.40 g (6.33 mmol, 63%); m.p.: 104–105 °C (Ref. [31] 106–107 °C); IR (KBr): ν = 3266, 2982, 1718, 1654, 1417, 1235, 1161, 1039, 1015 cm⁻¹; ¹H NMR: δ = 1.26 (3H, t, *J* = 7.3 Hz), 1.35 (3H, t, *J* = 7.3 Hz), 2.31 (3H, s), 2.42 (2H, t, *J* = 7.3 Hz), 2.70 (2H, t, *J* = 7.3 Hz), 4.13 (2H, q, *J* = 7.3 Hz), 4.32 (2H, q, *J* = 7.3 Hz), 9.19 (N-H, bs) ppm; ¹³C NMR: δ = 10.6, 14.1, 14.3, 22.0, 34.5, 60.1, 60.3, 72.5, 124.0, 126.5, 128.6, 160.4, 172.5 ppm.

2-Carboxy-5-(ethoxycarbonyl)-4-methyl-1H-pyrrole-3-propionic acid ethyl ester (10-2, C₁₄H₁₉NO₆)—As for the synthesis of **10-1**, 7.5 g sulfonyl chloride (4.5 cm³, 57 mmol) was added drop-wise to a stirring solution of 5 g pyrrole **11-2** (18.7 mmol) dissolved in 100 cm³ anhyd. ether, and cooled to 5 °C. The reaction and work-up proceeded as for **10-1** to yield ethyl ester **10-2**. Yield: 3.7 g (12.5 mmol, 67%); m.p.: 145–146 °C (Ref. [31] 146 °C); IR (KBr): ν = 3293, 2978, 2618, 1732, 1698, 1671, 1472, 1276, 1178, 1016 cm⁻¹; ¹H NMR ((CD₃)₂SO): δ = 1.23 (3H, t, *J* = 7.3 Hz), 1.38 (3H, t, *J* = 7.3 Hz), 2.30 (3H, s), 2.55 (2H, t, *J* = 7.3 Hz), 3.07 (2H, t, *J* = 7.3 Hz), 4.12 (2H, q, *J* = 7.3 Hz), 4.35 (2H, q, *J* = 7.3 Hz), 11.53 (N-H, bs), 12.86 (COOH, bs) ppm; ¹³C NMR ((CD₃)₂SO): δ = 9.7, 14.0, 14.2, 19.9, 34.6, 60.3, 60.7, 120.5, 122.9, 126.9, 131.4, 160.7, 164.9, 172.9 ppm.

Synthesis of (4.3)-homorubin (4-3)

2,2'-(1,4-Butanediyl)bis[5-[(3-ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-(Z)-1H-pyrrole-3-butanoic acid] [(4.3)-homorubin] (4-3, C₃₈H₅₀N₄O₆)—Homorubin dimethyl ester **4-3e** (30 mg, 0.046 mmol) was suspended in 5 cm³ N₂-purged CH₃OH and 15 cm³ THF and heated to reflux. Nitrogen-purged aq. NaOH (2 cm³, 1 M) was then added, and reflux was continued for 4 h. The mixture was cooled to room temperature, 5 cm³ H₂O was added, then the solvent was removed. Work-up continued as for **4-1** to give the desired **4-3**. Yield: 15 mg (0.037 mmol, 62%); m.p.: 225 °C (dec); IR (KBr): ν = 3356, 2934, 1667, 1634, 1257, 1362, 1246, 1173, 979, 680 cm⁻¹; NMR data in Table 5; UV-Vis data in Table 10.

2,2'-(1,4-Butanediyl)bis[5-[(3-ethyl-1,5-dihydro-4-methyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-(Z)-1H-pyrrole-3-butanoic acid] dimethyl ester [(4.3)-homorubin dimethyl ester] (4-3e, C₄₀H₅₄N₄O₆)—To a 50 cm³ round-bottom flask equipped with a magnetic stirrer and a heating mantle were added 300 mg bis-pyrrolbutane tetra-acid **5-3** (0.63 mmol), 377 mg 5-bromomethylene-3-pyrrolinone (**1**, 1.74 mmol), 18 cm³ CH₃OH, and 7 drops H₂O. The reaction and work-up proceeded as for **4-1e** to yield crude **4-3e** as yellow-green solid. Yield: 165 mg (0.23 mmol, 37%); m.p.: 260 °C (dec); IR (KBr): ν = 3356, 2935, 1734, 1664, 1625, 1508, 1246, 1173, 778, 678 cm⁻¹; ¹H NMR: ((CD₃)₂SO): δ = 1.04 (6H, t, *J* = 7.3 Hz), 1.55 (8H, brm), 1.74 (6H, s), 1.97 (6H, s), 2.24 (8H, m), 2.4–2.5 (8H, m) ppm; ¹³C NMR((CD₃)₂SO): δ = 8.1, 9.3, 14.9, 17.2, 23.0, 25.4, 26.0, 30.1, 32.87, 51.2, 97.9, 119.2, 122.0, 122.4, 122.6, 127.2, 134.1, 147.3, 172.0, 173.3

ppm; UV-Vis (acetonitrile): $\lambda_{\max}(\epsilon) = 399$ (54900) nm ($\text{mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$); UV-Vis (acetone): $\lambda_{\max}(\epsilon) = 408$ (61900) nm ($\text{mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$); UV-Vis (benzene): $\lambda_{\max}(\epsilon) = 402$ (60400), 417 (sh, 60000) nm ($\text{mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$); UV-Vis (CH_2Cl_2): $\lambda_{\max}(\epsilon) = 398$ (57000) nm ($\text{mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$); UV-Vis (CHCl_3): $\lambda_{\max}(\epsilon) = 405$ (59200) nm ($\text{mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$); UV-Vis ($(\text{CD}_3)_2\text{SO}$): $\lambda_{\max}(\epsilon) = 414$ (60000) nm ($\text{mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$); UV-Vis (CH_3OH): $\lambda_{\max}(\epsilon) = 415$ (63200) nm ($\text{mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$).

2,2'-(1,4-Butanediyl)bis(5-carboxy-4-methyl-1H-pyrrole-3-butanoic acid) (5-3, $\text{C}_{24}\text{H}_{32}\text{N}_2\text{O}_8$)—Bis-pyrrol butane **5-3e** (300 mg, 0.51 mmol) was dissolved in 10 cm^3 warm CH_3OH . Aqueous NaOH (20 cm^3 , 2 M) was added, and the solution was heated at reflux for 6 h. The reaction and work-up proceeded as for **5-1**, resulting in the desired **5-3**. Yield: 200 mg (0.379 mmol, 74%). It was characterized through the following methods and carried directly to the next step. M.p.: 140 °C (dec); ^1H NMR ($(\text{CD}_3)_2\text{SO}$): $\delta = 1.43$ (4H, brs), 1.51 (4H, dd, $J = 7.3$ Hz), 2.11 (6H, s), 2.14 (4H, t, $J = 7.3$ Hz), 2.24 (4H, t, $J = 7.3$ Hz), 2.43 (4H, brs), 10.82 (2N-H, brs), 11.85 (4H, COOH) ppm; ^{13}C NMR ($(\text{CD}_3)_2\text{SO}$): $\delta = 10.4, 22.9, 24.9, 26.1, 29.6, 33.2, 116.7, 119.7, 125.3, 134.4, 162.4, 174.5$ ppm.

2,2'-(1,4-Butanediyl)bis[5-(ethoxycarbonyl)-4-methyl-1H-pyrrole-3-butanoic acid] diethyl ester (5-3e, $\text{C}_{32}\text{H}_{48}\text{N}_2\text{O}_8$)—Bis-pyrrolbutadiyne **6-3e** (300 mg, 0.510 mmol) was dissolved in 200 cm^3 ethyl acetate followed by the addition of 30 mg platinum oxide. The flask was fitted with a hydrogen balloon and stirred until no fluorescence was observed (~24 h). The reaction was worked up as for **5-2e** to give a quantitative yield of the desired **5-3e**. Yield: 302 mg (0.510 mmol); m.p.: 122–123 °C; IR (KBr): $\nu = 3293, 2938, 1734, 1654, 1264, 1160, 1095 \text{ cm}^{-1}$; ^1H NMR: $\delta = 1.25$ (6H, t, $J = 7.3$ Hz), 1.34 (6H, t, $J = 7.3$ Hz), 1.60 (4H, brs), 1.73 (4H, m), 2.26 (6H, s), 2.28 (4H, t, $J = 7.3$ Hz), 2.39 (4H, t, $J = 7.3$ Hz), 4.12 (4H, q, $J = 7.3$ Hz), 4.24 (4H, q, $J = 7.3$ Hz), 8.56 (2N-H, bs) ppm; ^{13}C NMR: $\delta = 10.5, 14.3, 14.6, 23.4, 25.8, 33.8, 59.6, 60.2, 117.4, 121.0, 127.1, 133.5, 161.6, 173.5$ ppm.

2,2'-(1,4-Butadiynediyl)bis[5-(ethoxycarbonyl)-4-methyl-1H-pyrrole-3-butanoic acid] diethyl ester (6-3e, $\text{C}_{32}\text{H}_{40}\text{N}_2\text{O}_8$)—A mixture of 950 mg acetylenic pyrrole **7-3** (3.26 mmol) and 365 mg chloroacetone in 35 cm^3 dry benzene was added to a solution of 80 mg tetras(triphenylphosphine)palladium(0), 40 mg copper(I) iodide, and 650 mg triethylamine in 35 cm^3 dry benzene. The reaction and work-up proceeded as for **6-2e** to give the desired **6-3e** as light yellow crystals. Yield: 700 mg (1.2 mmol, 74%); m.p.: 124–125 °C; IR (KBr): $\nu = 3270, 2982, 2938, 2136, 1738, 1668, 1471, 1435, 1258, 1177, 1095, 1022, 867, 776 \text{ cm}^{-1}$; ^1H NMR: $\delta = 1.27$ (6H, t, $J = 7.3$ Hz), 1.38 (6H, t, $J = 7.3$ Hz), 1.85 (4H, m), 2.29 (6H, s), 2.33 (4H, t, $J = 7.2$ Hz), 2.52 (4H, t, $J = 7.2$ Hz), 4.14 (4H, q, $J = 7.3$ Hz), 4.36 (4H, q, $J = 7.3$ Hz), 9.23 (2N-H, bs) ppm; ^{13}C NMR: $\delta = 10.3, 14.3, 14.4, 24.2, 25.4, 33.6, 60.2, 60.4, 74.8, 78.5, 113.6, 121.2, 126.1, 132.7, 160.6, 173.3$ ppm; UV-Vis (CH_2Cl_2): $\lambda_{\max}(\epsilon) = 333$ (44500) nm ($\text{mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$); UV-Vis (CH_3OH): $\lambda_{\max}(\epsilon) = 330$ (48300) nm ($\text{mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$); UV-Vis ($(\text{CH}_3)_2\text{SO}$): $\lambda_{\max}(\epsilon) = 357$ (36500) nm ($\text{mol}^{-1} \text{ dm}^3 \text{ cm}^{-1}$).

5-(Ethoxycarbonyl)-2-ethynyl-4-methyl-1H-pyrrole-3-butanoic acid ethyl ester

(7-3, C₁₆H₂₁NO₄)—Ethyl ester **8-3** (1.70 g, 4.63 mmol) was dissolved in 22 cm³ dry THF. Anhydrous ethanol (45 cm³) and 960 mg potassium carbonate were then added, and the mixture was stirred for 4 h at room temperature. The reaction and work-up proceeded as for **7-2** to afford the desired ethyl ester **7-3**. Yield: 960 mg (3.29 mmol, 70%); m.p.: 102–103 °C; IR (KBr): ν = 3270, 2938, 2128, 1718, 1670, 1272, 1186, 1154, 1026 cm⁻¹; ¹H NMR: δ = 1.24 (3H, t, *J* = 7.3 Hz), 1.35 (3H, t, *J* = 7.3 Hz), 1.84 (2H, m), 2.27 (3H, s), 2.30 (2H, t, *J* = 7.3 Hz), 2.54 (2H, t, *J* = 7.3 Hz), 3.30 (1H, s), 4.11 (2H, q, *J* = 7.3 Hz), 4.31 (2H, q, *J* = 7.3 Hz), 9.01 (N-H, bs) ppm; ¹³C NMR: δ = 10.4, 14.3, 14.5, 24.0, 25.4, 33.7, 60.2, 60.3, 75.0, 82.5, 113.9, 120.1, 126.0, 130.4, 160.8, 173.5 ppm.

5-(Ethoxycarbonyl)-4-methyl-2-[(trimethylsilyl)ethynyl]-1H-pyrrole-3-butanoic acid ethyl ester (8-3, C₁₉H₂₉NO₄Si)

—As in the preparation of **8-2**, to a solution of 2.0 g ethyl ester **9-3** (5.09 mmol) in 60 cm³ diethylamine were added under N₂ 0.775 g (trimethylsilyl)acetylene (7.9 mmol), 65 mg bis(triphenylphosphine)palladium(II) chloride, and 36 mg copper(I) iodide. The reaction and work-up proceeded as for **8-2** to provide the desired ethyl ester **8-3**. Yield: 1.55 g (4.28 mmol, 84%); m.p.: 71–72 °C; IR (KBr): ν = 3278, 2965, 2148, 1734, 1675, 1250, 1176, 1097, 1026, 843 cm⁻¹; ¹H NMR: δ = 0.25 (9H, s), 1.24 (3H, t, *J* = 7.3 Hz), 1.35 (3H, t, *J* = 7.3 Hz), 1.85 (2H, m), 2.25 (3H, s), 2.30 (3H, t, *J* = 7.3 Hz), 2.53 (2H, t, *J* = 7.3 Hz), 4.11 (2H, q, *J* = 7.3 Hz), 4.30 (2H, q, *J* = 7.3 Hz), 8.81 (N-H, bs) ppm; ¹³C NMR: δ = -0.1, 10.3, 14.3, 14.5, 24.2, 25.3, 33.9, 60.2, 60.2, 95.8, 100.3, 115.0, 119.8, 126.1, 130.3, 160.8, 173.5 ppm.

5-(Ethoxycarbonyl)-2-iodo-4-methyl-1H-pyrrole-3-butanoic acid ethyl ester

(9-3, C₁₄H₂₀INO₄)—A solution of 3.38 g NaHCO₃ (40.25 mmol) in 8 cm³ H₂O was added at 65 °C to a stirred solution of 5.00 g **10-3** (16.1 mmol) in 80 cm³ CH₃OH. A solution of 4.09 g I₂ (16.1 mmol) and KI in 80 cm³ CH₃OH and 18 cm³ H₂O was added drop-wise during 1 h while the temperature was maintained at 60 °C. After stirring at 60 °C overnight, the solution was allowed to cool to room temperature. Then 300 cm³ H₂O was added to form a precipitate. The precipitate was collected by filtration, washed with water, dried under high vacuum, and recrystallized from CH₂Cl₂-light petroleum ether to afford ethyl ester **9-3**. Yield: 5.50 g (14.1 mmol, 87%); m.p.: 100–102 °C; IR (KBr): ν = 3254, 2936, 1718, 1684, 1419, 1255, 1185, 1154 cm⁻¹; ¹H NMR: δ = 1.25 (3H, t, *J* = 7.3 Hz), 1.35 (3H, t, *J* = 7.3 Hz), 1.78 (2H, brm), 2.30 (3H, s), 2.34 (3H, t, *J* = 7.3 Hz), 2.41 (2H, t, *J* = 7.3 Hz), 4.12 (2H, q, *J* = 7.3 Hz), 4.32 (2H, q, *J* = 7.3 Hz), 9.05 (N-H, bs) ppm; ¹³C NMR: δ = 10.8, 14.3, 14.5, 25.3, 25.9, 33.7, 60.2, 60.2, 72.2, 124.2, 126.7, 129.7, 160.6, 173.3 ppm.

2-Carboxy-5-(ethoxycarbonyl)-4-methyl-1H-pyrrole-3-butanoic acid ethyl ester

(10-3, C₁₅H₂₁NO₆)—Sulfonyl chloride (29 g, 159 mmol) was added drop-wise to a stirred solution of 15 g pyrrole **11-3** (53 mmol) dissolved in 280 cm³ anhyd. ether and cooled to 5 °C. Stirring was continued for 3 days at room temperature, and the ether was removed (rotovap). The remaining oil was taken into 210 cm³ dioxane, sodium acetate (15 g in 150 cm³ H₂O) was added, and the solution was stirred overnight. The solution was then extracted with ether (3×200 cm³), after which the ether was washed with sat. aq. Na₂CO₃. The aqueous layer was then cooled in an ice bath and acidified with nitric acid (20%) to

form a precipitate. The precipitate was collected by filtration, washed with H₂O, and dried in vacuo resulting in **10-3**. Yield: 8.7 g (28 mmol, 52%); m.p.: 140–141 °C; IR (KBr): ν = 3303, 2985, 2608, 1736, 1715, 1704, 1472, 1272, 1093, 1013, 913, 668 cm⁻¹; ¹H NMR ((CD₃)₂SO): δ = 1.14 (3H, t, *J* = 7.3 Hz), 1.28 (3H, t, *J* = 7.3 Hz), 1.68 (2H, m), 2.17 (3H, s), 2.22 (2H, t, *J* = 7.3 Hz), 2.68 (2H, t, *J* = 7.3 Hz), 3.99 (2H, q, *J* = 7.3 Hz), 4.21 (2H, q, *J* = 7.3 Hz), 11.63 (N-H, bs), 12.80 (COOH, bs) ppm; ¹³C NMR ((CD₃)₂SO): δ = 10.0, 14.4, 14.5, 23.5, 25.7, 33.5, 59.9, 60.1, 121.8, 127.9, 126.0, 129.8, 160.8, 162.0, 172.9 ppm.

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Fig. 1.

(A) Bilirubin-IX α in a porphyrin-like representation and (B) in its intramolecularly hydrogen-bonded planar projection. (C) The planar projection of intramolecularly hydrogen-bonded homorubins, where the integers m and n indicate the number of CH_2 groups in the linker at position 10 and in the alkanic acids, respectively. Hydrogen bonds are represented by dotted lines

Fig. 2.

(A) Intramolecularly hydrogen bonded bilirubin in its energetically favored ridge-tile shape, shown as interconverting enantiomeric conformations. (B) and (C) The [m,n]-homorubins: bilirubin analogs centrally homologated (m) at C(10) with carboxylic acid chains (n) lengthened or shortened: The interplanar angle (θ) of bilirubin is $\sim 100^\circ$; that of the homorubins depends on torsion angles A-F and hence the value of m and n. The (2.1)- and (4.1)-homorubins look more like (B). The (2.2), (2.3), (3.2), (4.2), and (4.3)-homorubins look more like (C). Hydrogen bonds are represented by dotted lines

A (2.1)

B (4.1)

C (2.2)

D (2.3)

E (3.2)

F (4.2)

G (4.3)

Fig. 3.

Ball and Stick representations of intramolecularly hydrogen-bonded minimum energy quasi-ridge tile conformations (A) and (B) of (2.1) and (4.1) homorubins, and conformations (C)-(G) of (2.2), (2.3), (3.2), (4.2), and (4.3) homorubins, respectively

Fig. 4. Variable temperature ¹H NMR of the N-H region (11.5–7.5 ppm) of homorubin **3-2** in CDCl₃. Temperatures (°C) are noted above the scans

Scheme 1.

Scheme 2.

Scheme 3.

Scheme 4.

Table 1

Potential energies (E_P) of hydrogen bonded (w/H-bonds), non-hydrogen bonded in the same conformation without (w/o H-bonds), number of hydrogen bonds, and the difference in energy between hydrogen-bonded and non-hydrogen-bonded ($E_P(w) - E_P(w/o)$) of (m.n)-homorubins, as computed by molecular mechanics and molecular dynamics^{a,b}

m	n	$E_P/\text{kJ mol}^{-1}$ w/H-bonds	Number of H-bonds	$E_P/\text{kJ mol}^{-1}$ w/o H-bonds	$E_P/\text{kJ mol}^{-1}$ $E_P(w) - E_P(w/o)$
2	1	57	6	29	-86
2	2	71	6	12	-83
2	3	63	6	-5	-58
3	1	84	6	12	-96
3	2	79	6	14	-93
3	3	68	6	35	-103
4	1	11	4	64	-75
4	2	55	4	27	-82
4	3	-77	6	1	-78

^a See Fig. 1B for intramolecularly hydrogen-bonded structure.

^b Calculated and determined using Sybyl version 6.0

Table 2

Hydrogen bond distances^a/Å of (m.n)-homorubins

(m.n)	OH...O=C Lactam	NH...O=C Lactam	NH...O=C Pyrrole	NH...O=C Pyrrole	NH...O=C Lactam	OH...O=C Lactam
(1.2) ^b	1.53	1.59	1.56	1.56	1.59	1.53
(2.1)	1.61	1.52	1.56	1.57	1.52	1.61
(2.2)	1.56	1.62	1.59	1.59	1.62	1.56
(2.3)	1.49	1.84	2.25	2.25	1.84	1.49
(3.2)	1.60	1.69	1.58	1.55	1.77	1.66
(4.1)	1.49	2.33	^c	^c	1.86	1.48
(4.2)	^c	1.53	1.57	1.59	1.54	^c
(4.3)	1.54	1.70	1.77	2.18	1.65	1.48

^aComputed by Syble vers 6.0 for the energy-minimized conformations.^bMesobilirubin-XIIIa.^cOutside H-bond distance.

Table 3

^{13}C and ^1H NMR chemical shifts^a and assignments of mesobilirubin-XIII α and the (2.1) and (4.1)-homorubins (2-1) and (4-1) with acetic acid chains

Atom Number ^b	MBR-XIII α m = 1, n = 1 ^c		(2.1)-Homorubin (2-1)		(4.1)-Homorubin (4-1)	
	Carbon	Proton	Carbon	Proton	Carbon	Proton
1, 19	172.6	–	171.8	–	171.94	–
2, 18	131.2	–	133.7	–	135.0	–
2 ¹ , 18 ¹	8.4	1.75	7.8	1.78	8.1	1.97
3, 17	147.4	–	146.7	–	147.3	–
3 ¹ , 17 ¹	17.7	2.44	17.0	2.51	17.2	2.46
3 ² , 17 ²	15.1	1.06	14.5	1.08	14.8	1.05
4, 16	123.4	–	122.5	–	122.8	–
5, 15	97.9	5.88	97.5	5.96	97.7	5.91
6, 14	122.7	–	122.1	–	121.9	–
7, 13	128.6	–	128.0	–	127.5	–
7 ¹ , 13 ¹	9.7	1.95	9.1	1.99	9.4	1.97
8, 12	114.7	–	113.8	–	113.6	–
8 ¹ , 12 ¹	29.8	3.11	29.5	3.21	29.9	3.22
9, 11	123.6	–	122.9	–	122.9	–
10(10a)	23.8	3.95	26.1	2.79	25.4	2.5
10b	–	–	–	–	29.8	1.53
COOH	174.5	11.87	172.8	12.06	173.1	11.98
21, 24	–	9.74	–	9.80	–	9.81
22, 23	–	10.28	–	10.24	–	10.24

^aChemical shifts recorded in ppm downfield from (CH₃)₄Si. Spectra from (CD₃)₂SO at 23 °C.

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

^cData from Ref. [15].

^{13}C and ^1H NMR chemical shifts^a and assignments of mesobilirubin-XIII α (n = 2) and homorubins (2-2), (3-2), and (4-2) with propionic acid chains

Table 4

Atom Number ^b	MBR-XIII α m = 1, n = 2 ^c		(2,2)-Homorubin (2-2)		(3,2)-Homorubin (3-2)		(4,2)-Homorubin (4-2)	
	Carbon	Proton	Carbon	Proton	Carbon	Proton	Carbon	Proton
1, 19	172.4	–	172.0	–	171.9	–	171.7	–
2, 18	130.8	–	133.0	–	133.8	–	133.8	–
2 ¹ , 18 ¹	8.5	1.75	8.1	1.78	8.1	2.22	7.8	1.98
3, 17	147.7	–	147.2	–	147.2	–	147.0	–
3 ¹ , 17 ¹	17.6	2.40	17.2	2.43	17.2	2.54	17.0	2.50
3 ² , 17 ²	15.3	1.06	14.8	1.08	14.9	1.04	14.5	1.02
4, 16	123.0	–	122.3	–	122.3	–	121.9	–
5, 15	98.2	5.92	97.8	5.94	97.7	5.89	97.4	5.89
6, 14	122.4	–	122.1	–	122.0	–	121.8	–
7, 13	128.3	–	127.6	–	127.4	–	127.3	–
7 ¹ , 13 ¹	9.6	1.97	9.3	2.00	9.3	1.74	9.0	1.74
8, 12	119.7	–	119.0	–	118.7	–	118.5	–
8 ¹ , 12 ¹	19.7	2.49	19.3	2.53	19.5	2.50	19.4	2.53
8 ² , 12 ²	34.8	2.40	34.9	2.45	35.3	2.25	35.4	2.45
9, 11	123.40	–	122.8	–	122.7	–	122.5	–
10(10a)	24.0	3.93	26.4	2.82	25.6	2.47	25.3	2.22
10b	–	–	–	–	31.0	1.75	29.9	1.53
COOH	174.5	11.87	174.2	11.95	174.2	12.06	173.7	12.02
21, 24	–	9.74	–	9.79	–	9.80	–	9.75
22, 23	–	10.28	–	10.17	–	10.17	–	10.15

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

^bSuperscripts refer to the carbons in the β -substituent chains, e.g., 2¹ is the first carbon attached to the ring on carbon C(2).

^cData from Ref. [15].

Table 5

^{13}C and ^1H NMR chemical shifts^a and assignments of mesobilirubin-XIII α ($n = 3$) and (2,3) and (4,3)-homorubins (2-3) and (4-3) with butyric acid side chains

Atom Number ^b	MBR-XIII α $m = 1, n = 3^c$		(2,3)-Homorubin (2-3)		(4,3)-Homorubin (4-3)	
	Carbon	Proton	Carbon	Proton	Carbon	Proton
1, 19	172.5	–	171.9	–	171.9	–
2, 18	130.7	–	132.9	–	134.0	–
2 ¹ , 18 ¹	8.5	1.75	9.0	1.78	8.1	1.74
3, 17	147.8	–	147.0	–	147.2	–
3 ¹ , 17 ¹	17.6	2.12	17.0	2.44	17.2	2.46
3 ² , 17 ²	15.3	1.06	14.5	1.08	14.8	1.04
4, 16	123.0	–	122.1	–	122.3	–
5, 15	98.2	5.93	97.9	5.96	97.7	5.89
6, 14	122.4	–	122.2	–	121.9	–
7, 13	128.3	–	127.5	–	127.2	–
7 ¹ , 13 ¹	9.6	1.98	7.8	1.97	9.3	1.98
8, 12	120.7	–	119.8	–	119.2	–
8 ¹ , 12 ¹	24.1	2.46	25.7	2.21	25.4	2.14
8 ² , 12 ²	23.4	1.30	22.9	1.4	23.0	1.53
8 ³ , 12 ³	33.8	1.99	33.1	2.08	33.2	2.47
9, 11	123.3	–	122.5	–	122.6	–
10(10a)	26.1	3.89	26.3	2.79	26.1	2.26
10b	–	–	–	–	30.1	1.53
COOH	174.9	11.50	174.2	12.03	174.4	11.96
21, 24	–	9.79	–	9.87	–	9.81
22, 23	–	10.28	–	10.15	–	10.14

^a Chemical shifts recorded in ppm downfield from (CH₃)₄Si. Spectra run in (CD₃)₂SO.

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

^c Data from Ref. [15].

Table 6

Comparison of mesobilirubin-XIII α and the homorubins' lactam and pyrrole N-*H* chemical shifts in CDCl₃ and (CD₃)₂SO^a

Analog	CDCl ₃		(CD ₃) ₂ SO	
	Lactam	Pyrrole	Lactam	Pyrrole
Mesobilirubin-XIII α ^b	10.57	9.15	9.72	10.27
(2.1)-Homorubin (2-1)	9.61	9.02	9.78	10.21
(2.2)-Homorubin (2-2) ^b	10.18	8.59	9.79	10.16
(2.3)-Homorubin (2-3) ^c	10.36	9.04	9.87	10.15
(3.2)-Homorubin (3-2)	9.67 (10.92) ^d	8.01 (10.17) ^d	9.80	10.17
(4.1)-Homorubin (4-1)	insoluble	insoluble	9.81	10.24
(4.2)-Homorubin (4-2)	9.79	8.22	9.79	10.15
(4.3)-Homorubin (4-3) ^{d,e}	10.36	8.54	9.81	10.14

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

^b Data from Ref. [14].

^c Data from Refs. [13] and [14].

^d These resonances disappear at 45 °C.

^e Due to poor solubility, the concentration was not determined.

Table 7HPLC retention times^a and TLC^b R_f values of homorubins compared to mesobilirubin-XIIIa

Pigment	Retention time/min	R _f
Mesobilirubin	14.85	0.89
2-1	19.54	0.58
2-2	12.83	0.63
2-3	15.37	0.92
3-2	9.67	0.36
4-1	9.96	0.32
4-2	8.47	0.39
4-3	10.81	0.45

^aFlow rate 1 cm³/min on an ion pairing ODS C-18 reversed phase analytical column (25 cm×4.6 mm) eluting with 0.1 M di-*n*-octylamine acetate in CH₃OH, 5% by vol. H₂O, pH 7.7.

^bCH₂Cl₂-CH₃OH (95:5 by vol.) eluent on silica gel

Table 8

Comparison of UV-Vis long wavelength absorption spectral data^a of mesobilirubin and homorubins with acetic acid chains^b

Solvent	MBR-XIIIa m = 1, n = 1 ^c		(2.1)-Homorubin 2-1		(4.1)-Homorubin 4-1	
	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}
Chloroform	423	57100	416	44300	410	47500
DMSO	424	49400	421	49800	413	51300
Methanol	422	50200	415	48800	409	49200
	396 ^s	45600	—	—	—	—
Acetonitrile	—	—	422	44600	405	45400
Acetone	—	—	411	48300	408	45100
Benzene	—	—	401	46500	413	45300

^a ϵ_{max} in $\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$ and λ_{max} in nm.

^b At room temperature for 1×10^{-5} M solutions.

^c Data from Ref. [15].

^s Shoulder or inflection determined by first and second derivatives.

Table 9

Comparison of UV-Vis long wavelength absorption spectral data^a of mesobilirubin and homorubins with propionic acid chains^b

Solvent	MBR-XIIIa m = 1, n = 2 ^c		(2,2)-Homorubin 2-2		(3,2)-Homorubin 3-2		(4,2)-Homorubin 4-2	
	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}
Chloroform	431 418 ^s	55400 53400	424	57000	437	69100	420	49900
DMSO	428 401 ^s	59100 50000	425	65700	424	61700	412	55000
Methanol	427 403 ^s	52100 45600	428	65100	429	67700	414	55700
Acetonitrile	420 396 ^s	56400 48900	418	57100	430	41400	410	47800
Acetone	424 422 ^s	55000 51500	407	50100	430	72700	410	52400
Benzene	453 418 ^s	55900 51600	423	61200	440	65200	418	52600

^a ϵ_{max} in $\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$ and λ_{max} in nm.

^b At room temperature for 1×10^{-5} M solutions.

^c Data from Ref. [37].

^s Shoulder or inflection determined by first and second derivatives.

Table 10

Comparison of UV-Vis long wavelength absorption spectral data^a of mesobilirubin and homorubins with butyric acid chains^b

Solvent	MBR-XIIIa m=1, n=3 ^c		(2,3)-Homorubin 2-3		(4,3)-Homorubin 4-3	
	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}
Acetonitrile			407	50300	412	46800
Acetone			411	49500	412	53700
Benzene			420	51800	420	52900
Chloroform	438	59700	421	51700	422	49400
DMSO	427	58200	405	56500	413	53400
	398 ^s	53000				
Methanol	428	52000	417	55300	416	55500
	398 ^s	54500				

^a ϵ_{max} in $\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1}$ and λ_{max} in nm.

^b At room temperature for 1×10^{-5} M solutions.

^c Data from Ref. [15].

^s Shoulder or inflection determined by first and second derivatives.

Table 11

Induced circular dichroism and UV-Vis spectra for mesobilirubin-XIII α and homorubin analogs in phosphate buffer at pH 7.4 with pigment : HSA ratio 2:1^a

Analog	Circular dichroism ^c			UV-Vis ^c
	$\epsilon_{\max} (\lambda_1)$	λ at $\epsilon = 0$	$\epsilon_{\max} (\lambda_2)$	$\epsilon_{\max} (\lambda_1)$
Mesobilirubin-XIII α ^b	+50 (440)	407	−44 (390)	44800 (434)
(2.1)-Homorubin m=2, n=1 (2-1)	+9.5 (443)	417	−11.8 (399)	47700 (420)
(2.2)-Homorubin m=2, n=2 (2-2)	+94 (433)	414	−80 (395)	52000 (411)
(2.3)-Homorubin m=2, n=3 (2-3)	+5.5 (405)	405	−5.7 (392)	47400 (434)
(3.2)-Homorubin m=3, n=2 (3-2)	−19 (437)	417	+18 (397)	57100 (428)
(4.1)-Homorubin m=4, n=1 (4-1)	−6 (432)	415	+8.4 (394)	44900 (420)
(4.2)-Homorubin m=4, n=2 (4-2)	−6 (436)	417	+8.7 (394)	53950 (421)
(4.3)-Homorubin m=4, n=3 (4-3)	−6.2 (435)	418	+8.6 (395)	52000 (420)

^aFor 1×10^{-5} M pigment solutions in a 1 cm cell at 25 °C.

^bData from Ref. [37].

^c ϵ and $\epsilon_{\max}$ in $\text{dm mol}^{-1} \text{ cm}^{-1}$ and λ in nm.

Table 12

Induced circular dichroism and UV-Vis spectra for mesobilirubin-XIII α and homorubins^a in CHCl₃ with a 1:300 pigment : quinine molar ratio

Analog	Circular dichroism ^c			UV-Vis ^c	
	$\epsilon_{\max}(\lambda_1)$	λ at $\epsilon = 0$	$\epsilon_{\max}(\lambda_2)$	$\epsilon_{\max}(\lambda_1)$	
Mesobilirubin-XIII α ^b	-49 (428)	402	+27 (383)	54100 (429)	
(2.1)-Homorubin (2-1)	+121 (434)	409	-80 (395)	47700 (421)	
(2.2)-Homorubin (2-2)	~0		~0	44700 (420)	
(2.3)-Homorubin (2-3)	~0		~0	63300 (426)	
(3.2)-Homorubin (3-2)	+20 (443)	419	-13 (400)	49300 (426)	
(4.1)-Homorubin (4-1)	+6.9 (443)	415	+8.4 (394)	43900 (417)	
(4.2)-Homorubin (4-2)	+4 (429)	410	3.6 (389)	52900 (428)	
(4.3)-Homorubin (4-3)	+6.4 (430)	407	-3 (390)	52100 (423)	

^aFor 1×10^{-5} M pigment solutions in a 1 cm cell at 25 °C.

^bData from Ref. [37].

^c ϵ and $\epsilon_{\max}$ in $\text{dm mol}^{-1} \text{ cm}^{-1}$ and λ in nm.