Published in final edited form as:

Monatsh Chem. 2014 July 1; 145(7): 1117–1135. doi:10.1007/s00706-014-1198-5.

Intramolecular Hydrogen Bonding and Linear Pentapyrrole Conformation

D. Timothy Anstine and

Department of Chemistry, Northwest Nazarene University, Nampa, ID 83686

David A. Lightner

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

David A. Lightner: lightner@unr.edu

Abstract

Three new linear pentapyrrole rubinoid analogs: 2,3,7,8,17,18,22,23-octamethyl-12,13-bis-(2-carboxyethyl)-1,10,15,24,25,27,28,29-octahydro-27*H*-pentapyrrin-1,24-dione and 2,3,8,12,13,17,22,23-octamethyl-7,18-bis-(2-carboxyethyl)-1,10,15,24,25,26,27,28,29-octahydro-27*H*-pentapyrrin-1,24-dione, and its 7,18-dihexanoic acid analog were synthesized, respectively, from 2,3,7,8-tetramethyl-(10*H*)-dipyrin-2-one, from 2,3,8-trimethyl-7-[2-(methoxycarbonyl)ethyl]-(10*H*)-dipyrinone, and 2,3,8-trimethyl-7-[5-(methoxycarbonyl)pentyl]-(10*H*)-dipyrinone. ¹³C NMR and ¹H NMR measurements in (CD₃)₂SO confirmed the pentapyrrole structures, while ¹H NMR data indicate intramolecular hydrogen bonding between the CO₂H and dipyrinone groups. Molecular mechanics modeling studies suggest stable U-shape conformations capable of encapsulating small planar aromatic molecules.

Keywords

Pyrroles; NMR; Hydrogen Bonding

Introduction

The hydrogen bonding capability of dipyrinones was first recognized in the structure (Fig. 1A) of bilirubin [1–3], the yellow pigment of jaundice [4], where intramolecular hydrogen bonding between a dipyrinone and carboxylic acid proved to be surprisingly effective in rendering bilirubins lipophilic, in dictating conformation [5–7], and even in organizing self-assembly [8, 9]. Dipyrinones are now known to be avid participants in hydrogen bonding [10–13], and absent an available carboxylic acid group, they tend to form intermolecularly hydrogen-bonded dimers [14]. The dipyrinone chromophore thus constitutes an effective molecular platform for molecular recognition [15]. Its proclivity to form intramolecular hydrogen bonds to a carboxylic acid group was explored in numerous linear tetrapyrroles [16–22], with chemically-designed dipyrinones [10–13, 23–25] and even with a few centrally expanded bilirubin analogs (Fig. 1B) [26–30], some of which (Fig.

1C) were designed to produce a concave conformation of possible use in sequestering planar guests. Though **B-1** of Fig. 1B exhibited most of the usual properties of bilirubin, the pigments of Fig. 1C proved to be too insoluble in nonpolar solvents, e.g., CHCl_3 , for ascertaining conformation and complexation [30]. Subsequent to that study, it was learned that tripyrrole **D-1** [31] and hemirubin **D-2** [32, 33] of Fig. 1D were intramolecularly hydrogen bonded, which suggests that the propionic acids of the pentapyrrole rubinoid **1** (Fig. 1E) might also engage in intramolecular hydrogen bonding. Accordingly, we extended our studies of dipyrinone-hydrogen bonding to the synthesis of pentapyrrole **1**, especially because molecular mechanics calculations predicted interesting folded conformations maintained by such hydrogen bonding.

Molecular modeling showed that **1** could populate two isoenergetic conformations. In each of these structures six intramolecular hydrogen bonds were possible (Fig. 2), with the overall shape of the molecule different from that of the ridge-tile conformation of bilirubin (Fig. 1A) and now being held in a “Z”-shape conformation (Fig. 3A) with the propionic acids and dipyrinones oriented toward opposite faces of the central pyrrole ring. The most intriguing aspect of the stereochemistry of **1**, however, was not the Z-shape but its ability to fold into yet a second type of conformation where the dipyrinones and the acid chains were oriented toward the same face of the central pyrrole. This orientation left **1** in a “U”-shape conformation with a cavity cleft (Fig. 3B), and it was 22.6 kJ mol^{-1} ($5.4 \text{ kcal mol}^{-1}$) more stable than the Z-shape. The greater predicted stability was apparently due to π -stacking interactions from the parallel chromophores as both configurations maintained the same matrix of six intramolecular hydrogen bonds (Fig. 3C).

These analyses prompted our expectation that other pentapyrroles might also adopt a U-shape cavity, and molecular modeling produced two new targets, **2** and **3** of Fig. 1E, with acid chains attached at pyrrole β -positions 7 and 18, as opposed to 12 and 13 in **C-2** of Fig. 1E. It was our expectation that **1-3** would have greater solubility than **C-1** and **C-2** in nonpolar solvents and would thus be more amenable to conformational analysis by ^1H NMR spectroscopy in CDCl_3 .

Results and discussion

Synthesis aspects

For the syntheses of **1-3**, we used the retrosynthetic approach found successful in the preparation of **C-1** and **C-2** of Fig. 1C [30] (Scheme 1). Thus, given appropriate 9H-dipyrinones and monopyrrole- α,α' -dialdehydes, one can see that verdin-like pentapyrroles **4-6** might be prepared by trifluoroacetic acid (TFA)-catalyzed condensation reactions similar to that first developed by Falk and Flödl [34]. The “verdins” would then be expected to be reduced by NaBH_4 to the corresponding rubins, **1-3**. The synthesis of **4** made use of the known tetramethyldipyrinone **7** [35] and the previously unknown monopyrrole **8**; whereas, the synthesis of **5** and **6** employed the known dimethylpyrrole dialdehyde **10** [36] and the previously unreported dipyrinones **9** and **11**. The preparation of **7** and **10** used a common intermediate, 3,4-dimethylpyrrole [37], which was converted to 3,4-dimethyl-3-pyrrolin-2-one using H_2O_2 in pyridine [37] and to its α -aldehyde [38] by a Vilsmeier formylation. Condensation of the aldehyde 3,4-dimethylpyrrole-2-aldehyde and the 3,4-

dimethyl-3-pyrrolin-2-one in aq. ethanolic KOH led to dipyrinone **7**. Conversion of the same pyrrole α -aldehyde to the α,α' -dialdehyde could not be accomplished directly by a second Vilsmeier reaction, probably due to deactivation of the pyrrole ring toward electrophilic attack by the aldehyde group already present. Although it could be shown that 3,4-dimethylpyrrole-2-carboxylic acid could be converted to the dialdehyde with triethyl orthoformate in TFA, the mono-aldehyde was protected by condensation with ethyl cyanoacetate then submitted to formylation via the Vilsmeier procedure. Deprotection afforded the desired dialdehyde **10** in good yield.

For the synthesis of **8**, we focused on preparing 3,4-bis(ethoxycarbonyl)pyrrole (**17**) and then building in formyl groups at both of the unsubstituted α -positions. A 13-step synthesis (Scheme 2) was initiated starting from succinic anhydride, as outlined in Scheme 2. Succinic anhydride was converted to its half ester by reaction with refluxing ethanol, and the half ester was immediately reacted with thionyl chloride to give β -carboethoxypropionyl chloride [39] as a clear liquid in 70% yield. Acylation of *t*-butyl acetoacetate [40] was followed by the addition of *p*-toluenesulfonic acid as a catalyst, and distillation under mild vacuum gave the desired ethyl 4,6-dioxoheptanoate (**12**) in 41% yield. Potassium fluoride-catalyzed Michael addition [41] of ethyl acrylate to **12** gave diethyl 5-acetyl-4-oxooctanedioate (**13**) in 89% yield. We used a 3:4 molar ratio of acrylate to heptanoate, which maximized formation of **13** and essentially completely reduced all side products. The resulting pure diethyl 5-acetyl-4-oxooctanedioate (**13**) was converted into a pyrrole by a modified Fisher-Knorr pyrrole synthesis to afford a 71% yield of **14** with the two required β -propionic ester groups. The target intermediate **17** required removal of both α -substituents from **14**, which was thought to be best accomplished by decarboxylation. And so the α -CH₃ was converted to α -CO₂H by perchlorination using SO₂Cl₂, followed by hydrolysis. This procedure resulted in partial saponification of esters, forming a mixture of three products; however, all remaining esters were saponified in aqueous ethanolic potassium hydroxide. After acidification, the desired pyrrole tetra-acid **15** was recovered in a moderate yield of 55% over two steps (the lower than expected yield was most likely due to incomplete perchlorination). The tetra-acid was surprisingly stable, turning pink only after several days at room temperature and exposure to air. The β -propionic acid groups were then selectively esterified by reaction in TFA with triethyl orthoformate [42] over a period of two days to afford diethyl ester **16** in excellent yield. Several unsuccessful attempts to decarboxylate the α -carboxyl groups of **16** by heating the solid directly, or in a Kugelrohr oven under vacuum up to 280 °C under nitrogen, were followed by successful decarboxylation following Woodward's method [43] in which an intimate mixture of the diacid-diester with sodium acetate and potassium acetate was heated to 140 °C (liquefaction occurs) under an atmosphere of nitrogen. This procedure gave pyrrole ester **17** in moderate yield (62%). Although α -H pyrroles can be formylated using ethyl or methyl orthoformate in TFA, formylation by Vilsmeier reactions was deemed more appropriate in order to avoid TFA-catalyzed intramolecular acylation of a β -propionate onto the α -carbon – as seen in earlier work [44]. Thus, a Vilsmeier reaction on **17** was carried out in dry ether with *N,N*-dimethylformamide and phosphorous oxychloride at 5 °C to give pyrrole **18** in 56% yield after hydrolysis. Attempts to introduce a second formyl group by repeating the Vilsmeier reaction on **18** failed.

Attachment of a second formyl group could be accomplished only after first protecting the formyl already present using ethyl 2-cyanoacetate catalyzed by diethylamine. The adduct **19** obtained, in 47% yield, was then converted to its diethyl ester to improve solubility for the second Vilsmeier reaction. Conversion to its dimethyl ester was attempted without success using diazomethane, however. Diazomethane possibly reacted with the activated vinyl group of the protected aldehyde as no desired product or starting material could be isolated after the reactions. Esterification of **19** proceeded smoothly, however, using TFA and triethyl orthoformate in a reaction allowed to proceed over a period of two days at room temperature to afford **20** in 98% yield. This conversion of **17** to **20** required three steps, including the final re-esterification. So a route was explored to preserve the ester groups throughout formylation and protection and thus save one reaction step. If the work-up of the Vilsmeier reaction of **17** was modified to the use of aqueous sodium acetate instead of aqueous sodium hydroxide, the ester groups were preserved, and **21** was obtained as a light brown viscous oil in 79% yield. Its formyl group was then protected with ethyl cyanoacetate to give **20** in 88% yield, and a much better yield over all. The vacant α -position of **20** was formylated using phosphorous oxychloride and *N,N*-dimethylformamide to give pyrrole **22** as a bright yellow viscous oil in 63% yield. Deprotection of the first introduced formyl group was accomplished using strong base, which also saponified the ester groups and gave α,α' -diformylpyrrole diacid **23** as a pink oil in 67% yield. Again, esterification using diazomethane failed to give the desired product, but with trimethyl orthoformate and TFA in methanol, the esterification proceeded smoothly to the target pyrrole **8** in 73% yield.

The synthesis of dipyrinones **9** and **11** required the previously described 3,4-dimethylpyrrolinone [37] and monopyrrole diesters **24** and **25** [45] as shown in Scheme 3. Oxidation of the α -CH₃ of **24** and **25** using ceric ammonium nitrate (CAN) [46] in acetic acid-H₂O-tetrahydrofuran (THF) afforded pyrrole aldehydes **26** and **27** in 95% yield. Formylpyrrole **26** was then stirred for 48 h at room temperature with 3,4-dimethyl-3-pyrrolin-2-one in the presence of 40% aqueous potassium hydroxide under nitrogen and in the dark. After acidic work-up, dipyrinone **28** was obtained as a yellow precipitate in 48% yield. Although Woodward et al. [43] had achieved decarboxylation of an α -CO₂H pyrrole, we were uncertain whether a similar decarboxylation might occur with **28**. Gratifyingly, we found that decarboxylation of the carboxy group at C(9) of **28** proceeded smoothly. Thus, sodium acetate trihydrate, potassium acetate, and diacid **28** were ground together in a mortar, and the intimate mixture was transferred to a round-bottom flask where it was smoothly decarboxylated at 130–140 °C. This new procedure gave pure **30** as a beautiful yellow precipitate in 70% yield. It was then esterified to its methyl ester using diazomethane to give the target dipyrinone **9** as a bright yellow solid in 72% yield after purification.

Similarly, the α -methyl group of pyrrole **25** was oxidized with CAN to give aldehyde **27**, in 96% yield, which was stirred for 48 h at room temperature with 3,4-dimethyl-3-pyrrolin-2-one in the presence of 40% aqueous potassium hydroxide under nitrogen and in the dark. After an additional four hours of reflux and acidic work-up, dipyrinone **29** was obtained as a bright yellow precipitate in 50% yield. Decarboxylation of **29**, as for **28**, at 135 °C gave pure **31** as a bright yellow precipitate in 95% yield. It was then esterified using diazomethane to give dipyrinone **11** as a bright yellow solid in 93% yield after purification.

With all five components in hand, two equivalents of the appropriate dipyrinone synthons were condensed with one equivalent of their complementary monopyrrole synthon dialdehyde to construct pentapyrindiones **4–6**. As per Falk and Flödl [34, 47] coupling the appropriate dialdehydes and α -free dipyrinones using TFA as solvent at 70 °C during two hours afforded high yields of pentapyrindiones as blue-green solids (Scheme 1). Although condensation reactions run at room temperature showed no product formation after 18 h, at elevated temperatures (ca. 70 °C) product formation was observed in minutes, and the reactions were usually complete in one hour [30]. The reactions were monitored by periodically quenching an aliquot of the reaction mixture with triethylamine and following the disappearance of the dipyrinones and the appearance of the product by thin layer chromatography (TLC). The reactions of dipyrinone **9** with dialdehyde **10**, dipyrinone **7** with dialdehyde **8**, and dipyrinone **11** with dialdehyde **10** were completed in 71%, 60%, and 91% yields, respectively.

The three verdin analogs (**4–6**) of Scheme 1 could then be reduced easily to their corresponding rubin dimethyl esters **1e**, **2e**, and **3e** using NaBH₄ in CH₃OH-THF [46] while sonicating the reaction mixtures (Scheme 1). Reductions of pentapyrindiones **5** and **6** with NaBH₄ proceeded slowly, with the color remaining blue-green, then gradually turning to red during three hours, and finally to yellow (after further addition of NaBH₄) during two hours. The yields of pentapyrrole rubin dimethyl esters **2e** and **3e**, after purification by silica-gel flash chromatography, were 97% and 94%, respectively. Pentapyrindione **4** reacted with NaBH₄ much more sluggishly, with the color remaining blue-green, then gradually turning to red during three hours, and finally to yellow (after further addition of NaBH₄) during five hours. The yield of pentapyrrole rubin dimethyl ester **1e**, after purification by silica-gel flash chromatography, was 70%. All three of these rubin dimethyl esters were soluble in chloroform and showed unusual ¹H NMR and UV-Vis spectral properties.

The target pentapyrrole rubin acids **2** and **3** were obtained by saponification of the corresponding rubin esters **2e** and **3e** with NaOH in methanol-tetrahydrofuran at reflux for three hours in yields of 84% and 64%, respectively. Again, due to its lack of solubility in methanol, pentapyrrole rubin acid **1e** required a much longer time (eight hours) to completely saponify. It finally yielded acid **1** in 81% yield.

Solution, chromatographic, and NMR spectral properties

The three pentapyrrole rubin diacids **1–3** exhibited very different solubility and chromatography properties. All are soluble in (CH₃)₂SO₄, as is **C-2**, a regio-isomer of **2**. Rubin acid **1** is slightly soluble in CHCl₃ but insoluble in CH₃OH; whereas, **2** is insoluble in CHCl₃ and only slightly soluble in CH₃OH. Hexanoic diacid **3** is slightly soluble in CHCl₃ and slightly soluble in CH₃OH. Typically, bilirubin analogs that are insoluble in CHCl₃ are usually soluble in CH₃OH, which is seen only for diacid **2** (and **C-2**). Diacid **1**, however, behaves very similar to bilirubin: slightly soluble in CHCl₃ and insoluble in CH₃OH, which suggests that diacid **1** might be expected to engage in intramolecular hydrogen bonding.

Further insight into polarity and therefore the preferred conformations of **1–3** were obtained from high performance liquid chromatography (HPLC). The reverse phase HPLC method,

developed by McDonagh (using methanol with di-*n*-octylamine acetate) [48] is very sensitive to small changes in polarity. For example, the greater polarity of **2** and **3** and similar polarity of **1**, as compared with mesobilirubin, are revealed by their reverse-phase retention times. Using McDonagh's buffer [47] (0.1 M di-*n*-octylamine acetate, in CH₃OH, pH 7.7, 5% H₂O) as eluent and mesobilirubin as a reference standard (retention time 17.4 min), its analog with ethyl groups replaced by methyls elutes faster (11.2 min), and **2** and **3** are comparably fast (11.3 and 11.5, respectively), but **1** elutes even slower than mesobilirubin (22.0 min). These data are consistent with intramolecular hydrogen bonding in **1**, and less so for **2** and **3**.

The assigned constitutional structures of the pentapyrrole rubinoid diacids **1–3** and **C-2** are consistent with their ¹³C NMR spectra data (Table 1), which show signals that correlate well with the expected molecular symmetry. The ¹³C chemical shifts of the component dipyrinones compare very favorably with those of permethylbilirubin [28], suggesting no strong perturbing interactions between the central pyrrolyl units and the flanking dipyrinones. The low solubility of **2** in CHCl₃ made it impossible to determine its ¹H NMR spectrum in CDCl₃, although spectra could be obtained in (CD₃)₂SO (Table 1).

Pentapyrrole rubin diacids **1** and **3** are soluble in CHCl₃ and thus ¹H NMR spectroscopy could be used as a probe of their conformations in CDCl₃. Table 2 shows a comparison of their chemical shifts with the bilirubin analog, 3,18-desvinyl-3,18-dimethylbilirubin-IX α (**D-3** of Fig. 1).

These data support the earlier suggestion from solubility and HPLC measurements that diacid **1** maintains intramolecular hydrogen bonding, somewhat comparable to that of a bilirubin. In bilirubins, the ¹H NMR N-H chemical shifts of the pyrrole and lactam have proven to be an excellent way to determine whether the dipyrinone units of bilirubins are involved in intramolecular hydrogen bonding [13, 19, 20, 27, 28]. Previous studies have shown that the carboxylic acid COOH appears near 13.6 and the lactam and pyrrole N-Hs appear near 10.6 and 9.2 ppm, respectively, in CDCl₃ solvent when the dipyrinone and carboxylic acid groups are intramolecularly hydrogen bonded, as shown in Figs. 1A, 2, and 3. In (CD₃)₂SO solvent, the distinctions seen for the dipyrinone N-Hs by ¹H NMR in CDCl₃ are altered, and all pyrrole N-H resonances appear near 10.2 ppm (Table 1). The data for diacid **1** follow the behavior of 3,18-desvinyl-3,18-dimethylbilirubin-IX α (**D-3**) in CDCl₃ and in (CD₃)₂SO, suggesting that it is involved in a similar sort of intramolecular hydrogen bonding. Another interesting result from the NMR data obtained in the non-polar solvent CDCl₃ is the relative positions of the central pyrrole N-Hs of **1** and **3**. For diacid **3**, it is 9.08 ppm and for **1** it is 6.91 ppm. These very different chemical shifts suggest that **1** and **3** adopt quite different conformations in non-polar solvents. In contrast, in the polar solvent (CD₃)₂SO, the chemical shifts (δ = 9.30 ppm for **1** and 9.25 ppm for **3**) are very similar (Table 1) and rather like the 9.08 ppm value seen for the pyrrole N-H of **3** in CDCl₃. The large up-field shielding of the central pyrrole proton of **1** in CDCl₃ suggests diamagnetic shielding due to the central pyrrole N-H lying above or below the flanking dipyrinone π -systems. The conformational picture that emerges for **3** is thus entirely consistent with the U-shape and Z-shape configurations shown in Fig. 3.

Information on the conformation of the pentapyrrole rubin dimethyl esters **1e–3e** can also be extracted from their NMR spectra. The N-H chemical shifts (Table 3) of **1e–3e** and **C-2e** in (CD₃)₂SO are, as expected, all very similar to the corresponding lactam and pyrrole N-H chemical shifts of the tetrapyrrole analog, the dimethyl ester (**D-3e**) of 3,18-desvinyl-3,18-dimethylbilirubin (**D-3**). In CDCl₃, the tetrapyrrole rubin dimethyl ester **D-3e** is thought to exist as an intermolecularly hydrogen bonded dimer, and its lactam N-H becomes slightly more deshielded than the pyrrole N-H (Table 3) [42, 49]. In pentapyrroles **1e–3e** and **C-2e**, the lactam and pyrrole N-H signals are even more strongly deshielded than in the tetrapyrrole ester, suggesting that intermolecular hydrogen bonding in these molecules has intensified.

Further insight into the conformation of the esters may be gained from an examination of the vicinal splittings of the –CH₂– carboxylic acid ester groups (Table 4). The C(10) –CH₂– of **D-3e** is a singlet, but in **1e–3e** and **C-2e** the hydrogens are non-equivalent, and large geminal splittings are observed. In addition, the –CH₂–CH₂– propionic ester segments of **1**, **2**, and **C-2e** and hexanoic ester segment of **3e** are apparently much less flexible than in **D-3e** and show well-defined sets of doublets of doublets of doublets in **1** and **C-2e** and doublets of triplets in **1e** and, from the ε-methylene (C(7¹) and C(18¹)) of **3**. The data may be contrasted to the simple triplet in **D-3e**. The splittings in the propionic and hexanoic segments are more like those seen in bilirubin (ddd for ABCX) than its esters (t for A₂B₂) and are consistent with a picture where motion in the propionic acid groups of the esters is restricted by intramolecular hydrogen bonding.

The verdinoid pentapyrrindione dimethyl esters **4e–6e** exhibit properties very similar to those of mesobiliverdin-XIIIa dimethyl ester. They are soluble in chloroform and insoluble in methanol. Their proton and carbon NMRs, consistent with their structures and with the NMR of the decamethyl analog of Falk and Flödl [34] are relatively simple due to symmetry [48]. In the ¹H NMR in CDCl₃ they have two N-H signals at 9.1, 8.9, and 9.2 ppm (2 hydrogen integration), as seen for **4**, **5**, and **6**, respectively, and at 12.3, 12.0, and 12.3 ppm (1 hydrogen integration), respectively. These data clearly indicate that the central N-H is hydrogen-bonded to its neighboring pyrroles and the lactam N-Hs are not hydrogen-bonded to their neighboring pyrrolenes. Complete conjugation along the pentapyrrindione backbones of **4–6** is indicated by the characteristic peaks around 7 ppm in the ¹H NMR and around 113 ppm in the ¹³C NMR, consistent with the (9Z,15Z) *exo*-cyclic double bonds as determined by Falk and Flödl for the decamethyl pentapyrrindione [34].

Further insight into the preferred conformations of the pentapyrrole rubinoids was provided by an analysis of the diacids using UV-Vis spectroscopy (Table 3). The UV-Vis spectra (Table 5) of yellow diacids **1–3** and **C-2** were found to be solvent dependent. Pentapyrrole rubinoid acids **2**, **3**, and **C-2** show blue-shifted (hypsochromic) spectra in non-polar solvents and red-shifted (bathochromic) spectra in polar solvents. These data suggest a conformational change from an intramolecular hydrogen-bonded conformation in non-polar solvents to a more open conformation with less hydrogen bonding in polar solvents. The data from **1** differ and are more consistent with the behavior of bilirubin and mesobilirubin, where smaller solvent shifts and a bathochromic shift in non-polar solvents obtain. The data

are consistent with **1** adopting an entirely different conformation from those of **2**, **3**, or **C-2**, which probably adopt rather similar conformations.

In contrast, the pentapyrrole yellow-orange rubinoid methyl esters all show relatively similar wavelength shifting patterns in solvents of varying polarities. The least polar solvents (lower dielectric constant) are expected to promote intramolecular hydrogen bonding; whereas, the more polar solvents (higher dielectric constant) should interfere with intramolecular hydrogen bonding and support monomeric species, with solvent intermolecular hydrogen bonding to the pigments. Unlike the acids (Table 5), this trend is repeated throughout the UV-Vis spectra for all four esters. The non-polar solvents benzene and chloroform show consistent shifts around 390 nm, while the polar solvents DMF ((CH₃)₂NCHO) and DMSO ((CH₃)₂SO) show consistent shifts above 400 nm (Table 6).

The verdinoid molecules have interesting and unusual UV-Vis spectroscopic properties (Table 7). This is obvious by visual examination as all three new pentapyrindiones are green. UV-Vis spectroscopy of the three new pentapyrindiones revealed two characteristic [47] absorbances at around 730 nm and 460 nm in CHCl₃. These two wavelengths of light correspond to the primary colors blue and yellow, respectively, and are the source of the green color of **4-6**.

Circular dichroism

When a compound absorbs UV-Vis 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 (ICD) results. An example of the latter can be seen with bilirubin [51], which exists as two interconverting conformational enantiomers with (*M*) and (*P*) helicities. Since these two conformations are isoenergetic enantiomers, bilirubin is racemic and does not show a CD (because no discrimination between the (*M*) and (*P*) helicities exists). However, discrimination (chirality) can be induced by shifting the (*M*) and (*P*) conformational equilibrium in bilirubin following binding to a protein. In this situation, an ICD can be measured. Although many proteins can induce CD in bilirubin, human serum albumin (HSA) and bovine serum albumin (BSA) have been studied most often, and they tightly bind bilirubin [52, 53].

Pentapyrrole rubin diacids **1**, **2**, **3**, and **4**, like bilirubin, are achiral and do not show a CD. However, in the presence of aq. HSA in a 2-to-1 protein-to-pigment ratio, CD from the pigment can be detected (Table 8).

The presence of bisignate curves indicates that the diacids probably have fixed chiral conformations when bound to HSA and BSA. When diacid **2** is bound to HSA and BSA, it behaves similarly to mesobilirubin. When diacids **1**, **3**, and **C-2** are bound to HSA, the signs of their CDs are opposite and when bound to BSA, the signs of their CDs are the same as mesobilirubin. The origin of the optical activity comes from the pigment adopting a chiral conformation selected at the binding site on the protein. The observed bisignate CDs come from exciton coupling of the two electric dipole transitions: one from each of the pigment's

twin dipyrinone chromophores, viz., those from the long wavelength UV-Vis excitation near 410 nm. The protein acts as an enantioselective binding agent and constrains the pigment to adopt a chiral conformation.

Conformational analysis

Given that the U-shape of pentapyrrole **1** was favored energetically over the Z-shape (Fig. 3), the geometry of the U-shape conformer cleft (Fig. 4A) was probed to learn if it could accommodate a guest molecule.

The global minimum configuration optimized π -stacking by orienting the chromophores 3.0 Å away from one another [54, 55]. If a guest molecule were going to be bound inside the cleft, this distance needed to be ~ 6 Å [52, 53]. Using molecular modeling, it was discovered that the distance between the chromophores could be increased to 5.8 Å and that a cleft did open in a favorable fashion to accompany guest inclusion, as in Fig. 4B. Several polycyclic aromatic hydrocarbon (PAH) guests were modeled as a probe of the dimensions of the pocket and the binding ability of the pentapyrrole, which showed the unique ability of pentapyrrole **1** to bind relatively large and flat aromatic guest molecules, such as naphthalene, anthracene, phenanthracene, methoxsalen, 5-fluoroacid, etc., by expanding its cleft and still retain its moiety of six hydrogen bonds. Yet despite the potential exhibited for **1** to act as a host for methoxsalen and pyrene, ^1H NMR measurements designed to detect complexation [54] in CDCl_3 indicated no binding. Here as well as with **2** or **3** (predicted binding cleft 5.8 Å), the dimension of the central pyrrole ring that serves as the floor of the potential binding well is simply too narrow (~ 5.4 Å), and a wider gap is predicted to be required. Guests with non-planar shapes (cyclophosphamide, cytarabine, and ifosfamide) did not maximize the π -stacking in the cleft and therefore had lower binding energies.

Concluding comments

New pentapyrrole rubinoids **1–3** synthesized from monopyrroles were designed for intramolecular hydrogen bonding, as predicted by molecular dynamics calculations using Sybyl. Such intramolecular hydrogen-bonded structures may adopt Z-shape and U-shape conformations (Fig. 2A). The former consist of isoenergetic enantiomeric conformers (Fig. 2). The latter, which is predicted to be more stable, is a conformation with a cleft (Fig. 3B and 3C) potentially capable of trapping small planar molecules such as 5-fluorouracil (Fig. 4B). However, we could detect no bonding between the U-shape conformer and the fluorouracil.

Experimental

All nuclear magnetic resonance (NMR) spectra were obtained on a Varian 500 MHz (^1H) and 125 MHz (^{13}C), or General Electric QE-300 MHz instruments in deuteriochloroform (CDCl_3) unless otherwise indicated. Chemical shifts were reported in ppm referenced to the residual chloroform proton signal at 7.26 ppm and ^{13}C signal at 77.23 ppm unless otherwise noted. Melting points were taken on a Thomas-Hoover capillary apparatus. Elemental analyses, obtained from Desert Analytics, Tucson, Arizona, were within $\pm 0.4\%$ of the calculated values. High resolution determinations of molecular ions were obtained from the

Proteomics Center of the University of Nevada. All ultraviolet-visible (UV-Vis) spectra were recorded on a Perkin-Elmer λ -12 spectrophotometer and all infrared spectra were obtained on a Perkin-Elmer 1600 FT-IR instrument. Mass spectral data were obtained on a Hewlett-Packard 5970 mass selective detector unless otherwise indicated. HPLC analyses were carried out on a Perkin-Elmer Series 4 high performance liquid chromatography with an LC-95 UV-Vis spectrophotometric detector (set at 410 nm) equipped with a Beckman-Altex ultrasphere-IP 5 μ m C-18 ODS column (25 $\times$ 0.46 cm) and a Beckman ODS precolumn (4.5 $\times$ 0.46 cm). The flow rate was 1.0 cm³/min, and the elution solvent was 0.1 M di-*n*-octylamine acetate in 5% aqueous methanol (pH 7.7, 31 °C). Circular dichroism spectra were recorded on a Jasco J-40 spectrometer. Analytical thin layer chromatography (TLC) was carried on J.T. Baker silica gel IB-F plates (125 μ m layer). Flash column chromatography was carried out using 60–200 mesh silica gel (M. Woelm, Eschwege). For final purification, radial chromatography was carried out on 1 or 2 mm thick rotors of Merck silica gel PF₂₅₄ with calcium sulfate binder, preparative layer grade, using a Chromatotron (Harrison Research, Inc., Palo Alto, CA). All solvents were reagent grade obtained from Fisher-Acros.

The spectral data were obtained in spectral grade solvents (Aldrich or Fisher). Succinic anhydride, diethyl malonate, ethyl cyanoacetate, *tert*-butyl acetoacetate, ethyl 6-bromohexanoate, ethyl acrylate, ceric ammonium nitrate (CAN), and triethyl and trimethyl orthoformate were from Aldrich Chemical Co. Pyrroles **24** and **25** were synthesized according to literature methods [45].

(4Z,20Z)-2,3,7,8,17,18,22,23-Octamethyl-12,13-bis-(2-carboxyethyl)-1H,10H,15H,24H,25H,26H,27H,28H,29H-pentapyrrin-1,24-dione (1, C₃₈H₄₇N₅O₆)

In a 100 cm³ round-bottom flask equipped with a heating mantle and reflux condenser, 15 mg dimethyl ester **1e** (0.022 mmol) was dissolved in 15 cm³ THF with 7 cm³ CH₃OH. Sodium hydroxide (1 cm³, 1 M) was added, and the solution was heated at reflux for 8 h under N₂ and in the dark. The solution was cooled and the solvents were removed (rotovap). Water (10 cm³) was added to redissolve the salts, and the solution was acidified to pH 5 with 1.0 M hydrochloric acid. The resulting precipitate was allowed to stir in the acid solution for 20 min and then was collected by centrifugation. The yellow-green pentapyrrole rubin was washed with H₂O (3 $\times$ 20 cm³), re-centrifuging each time, and then collected by filtration. Yield: 12 mg (0.017 mmol, 81%); m.p.: 278–280 °C; IR (KBr): ν = 3423, 2918, 2861, 1655, 1637, 1388, 1349, 1266, 1174, 943, 691 cm⁻¹; UV-Vis data in Table 5; ¹H and ¹³C NMR in Table 1.

(4Z,20Z)-2,3,8,12,13,17,22,23-Octamethyl-7,18-bis-(2-carboxyethyl)-1H,10H,15H,24H,25H,26H,27H,28H,29H-pentapyrrin-1,24-dione (2, C₃₈H₄₇N₅O₆)

In a 100 cm³ round-bottom flask equipped with a heating mantle and reflux condenser, 20 mg dimethyl ester **2e** (0.03 mmol) was dissolved in 10 cm³ THF with 10 cm³ CH₃OH and saponified as for **1**. Yield: 17 mg (0.025 mmol, 83%); m.p.: 280–285 °C; IR (KBr): ν = 3396, 2921, 1663, 1637, 1443, 1390, 1267, 1167, 944, 691 cm⁻¹; UV-Vis data in Table 5; ¹H and ¹³C NMR in Table 1. (4Z,20Z)-2,3,8,12,13,17,22,23-Octamethyl-7,18-bis-(5-carboxypentyl)-1H,10H,15H,24H,25H,26H,27H,28H,29H-pentapyrrin-1,24-dione (**3**,

$C_{43}H_{59}N_5O_6$) In a 100 cm³ round-bottom flask equipped with a heating mantle and reflux condenser, 9 mg dimethyl ester **3e** (0.012 mmol) was dissolved in 10 cm³ THF with 7 cm³ CH₃OH and saponified as for **1**. Sodium hydroxide (1 cm³, 1 M) was added, and the solution was heated at reflux for 3 h under N₂ and in the dark. The solution was then cooled and the solvents were removed (roto-vap). Water (10 cm³) was added to redissolve the salts, and the solution was acidified to pH 5 with 1.0 M HCl. The resulting precipitate was allowed to stir in the acid solution for 20 min and then was collected by centrifugation. The yellow-green pentapyrrole rubin was washed with water (3 × 20 cm³), re-centrifuging each time, and then collected by filtration. Yield: 6 mg (0.007 mmol, 64%); m.p.: 226 °C (dec); UV-Vis data in Table 5; ¹H NMR and ¹³C NMR in Table 1.

(4Z,20Z)-2,3,7,8,17,18,22,23-Octamethyl-12,13-bis-[2-(methoxycarbonyl)ethyl]-1H,10H,15H,24H,25H,26H,27H,28H,29H19 pentapyrrin-1,24-dione (1e, C₄₀H₅₁N₅O₆)

Pentapyrrole verdin **4** (15 mg, 0.022 mmol) was dissolved in 20 cm³ hot THF in a 125 cm³ Erlenmeyer flask and placed in a sonicator bath under a nitrogen blanket. Methanol (10 cm³) was added, followed by 10 mg NaBH₄, and the reaction was sonicated for 3 h. Additional sodium borohydride (10 mg) was added, and the reaction was sonicated for another 5 h. Sonication was stopped and hydrochloric acid was added until the yellow mixture's pH was 3. The mixture was taken up in 50 cm³ CH₂Cl₂ and washed with H₂O (3 × 50 cm³). The organic solution was dried over sodium sulfate and filtered, and the CH₂Cl₂ was removed (roto-vap). The yellow product **5** was purified by flash chromatography (TLC on silica gel deactivated with 10% H₂O) using CH₂Cl₂-CH₃OH 99:1 (by vol) eluent. Yield: 11 mg (70%); m.p.: 263–265 °C; IR (KBr): ν = 3368, 2920, 1736, 1664, 1636, 1509, 1458, 1364, 1269, 1176, 944, 691 cm⁻¹; UV-Vis data in Table 6; ¹H NMR (CDCl₃): δ = 1.18 (6H, s), 1.10 (6H, s), 1.93 (6H, s), 2.06 (6H, s), 2.46 (2H, ddd, J = 5.5 Hz, 10.5 Hz, 15.0 Hz), 2.51 (2H, ddd, J = 9.3 Hz, 6.5 Hz, 15.0 Hz), 2.79 (2H, ddd, J = 14.3 Hz, 6.5 Hz, 10.5 Hz), 2.92 (2H, ddd, J = 14.3 Hz, 9.3 Hz, 5.5 Hz), 3.66 (6H, s), 3.81 (2H, d, J = 15.5 Hz), 3.89 (2H, d, J = 15.5 Hz), 5.88 (2H, s), 9.58 (1H, s), 10.37 (2H, s), 11.02 (2H, s) ppm; ¹³C NMR (CDCl₃): δ = 8.1, 9.4, 10.1, 10.3, 20.8, 23.2, 37.0, 51.9, 100.6, 114.9, 116.0, 122.6, 124.0, 124.8, 125.1, 128.8, 133.8, 142.0, 172.8, 174.3 ppm.

(4Z,20Z)-2,3,8,12,13,17,22,23-Octamethyl-7,18-bis-[2-(methoxycarbonyl)ethyl]-1H,10H,15H,24H,25H,26H,27H,28H,29H3 pentapyrrin-1,24-dione (2e, C₄₀H₅₁N₅O₆)

Pentapyrrole verdin **5** (20 mg, 0.03 mmol) was dissolved in 20 cm³ hot THF in a 125 cm³ Erlenmeyer flask, placed in a sonicator bath under a nitrogen blanket, and reduced with NaBH₄ as for **1e**. Yield: 20 mg (99%); m.p.: 250–253 °C; IR (KBr): ν = 3376, 2919, 1738, 1662, 1637, 1439, 1271, 1175, 944, 691 cm⁻¹; UV-Vis data in Table 6; ¹H NMR (CDCl₃): δ = 1.17 (6H, s), 1.95 (6H, s), 2.08 (6H, s), 2.11 (2H, dt, J = 17 Hz, 7.5 Hz), 2.17 (2H, dt, J = 17 Hz, 7.5 Hz), 2.47 (2H, dt, J = 7.5 Hz), 2.52 (2H, dt, J = 15 Hz, 7.5 Hz), 3.66 (6H, s), 3.78 (2H, d, J = 15.5 Hz), 3.87 (2H, d, J = 15.5 Hz), 5.92 (2H, s), 9.35 (1H, s), 10.47 (2H, s), 11.15 (2H, s) ppm; ¹³C NMR (CDCl₃): δ = 8.5, 9.2, 10.2, 10.4, 20.6, 23.2, 36.0, 52.1, 100.3, 112.3, 115.2, 122.2, 124.2, 124.3, 127.8, 129.10, 134.8, 142.2, 174.0, 174.5 ppm.

(4Z,20Z)-2,3,8,12,13,17,22,23-Octamethyl-7,18-bis-[5-(methoxycarbonyl)pentyl]-1H,10H,15H,24H,25H,26H,27H,28H,29H-18 pentapyrrin-1,24-dione (3e, C₄₅H₆₃N₅O₆)

Pentapyrrole verdin **6** (10 mg, 0.013 mmol) was dissolved in 15 cm³ hot THF in a 125 cm³ Erlenmeyer flask and placed in a sonicator bath under a nitrogen blanket and reduced with NaBH₄ as for **1e**. Yield: 9 mg (94%); m.p.: 233–237 °C; IR (KBr): ν = 3447, 2919, 1737, 1663, 1637, 1459, 1267, 1176, 942, 691 cm⁻¹; UV-Vis data in Table 6; ¹H NMR (CDCl₃): δ = 1.70 (6H, s), 1.39 (4H, p, *J* = 7.5 Hz), 1.45–1.52 (4H, m), 1.67 (4H, p, *J* = 7.5 Hz), 1.92 (6H, s), 1.93 (6H, s), 2.09 (6H, s), 2.32 (4H, t, *J* = 7.5 Hz), 2.43 (2H, dt, *J* = 8 Hz, 14 Hz), 2.48 (2H, dt, *J* = 8 Hz, 14 Hz), 3.65 (6H, s), 3.80 (2H, d, *J* = 15.5 Hz), 3.88 (2H, d, *J* = 15.5 Hz), 5.85 (2H, s), 9.37 (1H, s), 10.48 (2H, s), 11.17 (2H, s) ppm; ¹³C NMR (CDCl₃): δ = 8.5, 9.4, 10.2, 10.3, 23.2, 25.0, 25.4, 29.6, 31.7, 34.5, 51.9, 100.5, 112.2, 115.2, 122.2, 124.2, 124.3, 128.7, 130.1, 134.6, 141.8, 174.4, 174.6 ppm.

(4Z,9Z,15Z,20Z)-2,3,7,8,17,18,22,23-Octamethyl-12,13-bis[2-(methoxycarbonyl)ethyl]-1H,24H,25H,27H,29H-pentapyrrin-1,24-dione (4, C₄₀H₄₇N₅O₆)

In a 25 cm³ round-bottom flask 73 mg dipyrnone **7** (0.34 mmol) [42] and 45 mg diethyl 2,5-diformyl-1H-pyrrole-3,4-dipropionate (**8**, 0.16 mmol) were intimately co-mixed, and 10 cm³ trifluoroacetic acid was added dropwise. The flask was equipped with a reflux condenser, and the solution was stirred magnetically and blanketed with N₂. The reaction mixture was heated to 70 °C for 2.5 h in the dark, then cooled to 5 °C. Triethylamine (11 cm³) was added slowly to neutralize the reaction, and the solution was taken up in 50 cm³ CH₂Cl₂, washed with H₂O (3 × 20 cm³), and dried over anhyd. MgSO₄. The MgSO₄ was removed by filtration and the CH₂Cl₂ was removed (roto-vap) to give a green-brown solid. The compound was purified by flash chromatography (TLC silica gel deactivated with 10% H₂O) using CH₂Cl₂-CH₃OH 99:1 (by vol) as solvent to give a greenish yellow solid. Yield: 66 mg (0.096 mmol, 60%); IR (KBr): ν = 3447, 2919, 1737, 1702, 1649, 1396, 1226, 1143, 1096 cm⁻¹; UV-Vis (CHCl₃): λ_{max} (ϵ) = 320 (54700), 444 (21200), 712 (7800) nm (M⁻¹ cm⁻¹); UV-Vis (CH₃OH): λ_{max} (ϵ) = 321 (56200), 442 (25700), 710 (7500) nm (M⁻¹ cm⁻¹); ¹H NMR (CDCl₃): δ = 1.79 (6H, s), 1.95 (6H, s), 2.01 (6H, s), 2.62 (4H, t, *J* = 7.8 Hz), 3.03 (4H, t, *J* = 7.8 Hz), 3.69 (6H, s), 5.61 (2H, s), 6.81 (2H, s), 8.89 (2H, s), 12.01 (1H, s) ppm; ¹³C NMR (CDCl₃): δ = 8.9, 9.9, 10.2, 10.4, 20.1, 36.6, 52.3, 97.0, 112.8, 130.1, 131.6, 134.4, 136.0, 139.9, 140.1, 143.6, 155.3, 169.3, 171.3, 173.5 ppm.

(4Z,9Z,15Z,20Z)-2,3,8,12,13,17,22,23-Octamethyl-7,18-bis[2-(methoxycarbonyl)ethyl]-1H,24H,25H,27H,29H-pentapyrrin-1,24-dione (5, C₄₀H₄₇N₅O₆)

In a 10 cm³ round-bottom flask 50 mg dipyrnone **9** (0.18 mmol) and 15 mg 3,4-dimethyl-1H-pyrrole-2,5-dicarbaldehyde (**10**, 0.09 mmol) [43] were intimately mixed, and 5 cm³ TFA was added dropwise to effect the condensation, as for **4**. Yield: 44 mg (0.064 mmol, 71%) as a greenish yellow solid; m.p.: 236–238 °C; IR (KBr): ν = 3425, 2919, 1737, 1702, 1631, 1596, 1437, 1396, 1343, 1220, 1167, 1079, 955, 750 cm⁻¹; UV-Vis (CHCl₃): λ_{max} (ϵ) = 320 (55600), 456 (19300), 736 (8800) nm (M⁻¹ cm⁻¹); UV-Vis (CH₃OH): λ_{max} (ϵ) = 318 (60000), 451 (18800), 730 (8700) nm (M⁻¹ cm⁻¹); ¹H NMR (CDCl₃): δ = 1.78 (6H, s), 2.00 (6H, s), 2.17 (6H, s), 2.21 (6H, s), 2.47 (4H, t, *J* = 7.8 Hz), 2.68 (4H, t, *J* = 7.8 Hz), 3.68 (6H, s), 5.59 (2H, s), 6.75 (2H, s), 9.14 (2H, s), 12.33 (1H, bs) ppm; ¹³C NMR

(CDCl₃): δ = 8.9, 9.7, 10.0, 10.2, 20.4, 34.2, 52.1, 97.0, 113.8, 129.9, 130.0, 136.3, 137.1, 140.2, 140.9, 143.5, 154.7, 168.0, 171.5, 174.1 ppm.

(4Z,9Z,15Z,20Z)-2,3,8,12,13,17,22,23-Octamethyl-7,18-bis-[5-(methoxycarbonyl)pentyl]-1H,24H,25H,27H,29H-pentapyrrin-1,24-dione (6, C₄₆H₅₉H₅O₆)

In a 25 cm³ round-bottom flask 35 mg dipyrinone **11** (0.11 mmol) and 8 mg 3,4-dimethyl-1H-pyrrole-2,5-dicarbaldehyde (**10**, 0.053 mmol) were intimately mixed, 5 cm³ TFA was added dropwise, and the condensation reaction was conducted as for **5**. Yield: 37 mg (0.048 mmol, 91%) as a greenish yellow solid; m.p.: 204–206 °C; IR (KBr): ν = 3434, 2931, 2861, 1737, 1704, 1625, 1596, 1437, 1390, 1337, 1220, 1143, 1096, 997, 756, 550 cm⁻¹; UV-Vis (CHCl₃): λ_{max} (ϵ) = 320 (57800), 454 (19500), 733 (8900) nm (M⁻¹ cm⁻¹); UV-Vis (CH₃OH): λ_{max} (ϵ) = 318 (61000), 450 (18400), 728 (8600) nm (M⁻¹ cm⁻¹); ¹H NMR (CDCl₃): δ = 1.43–1.45 (br m), 1.48 (4H, p, J = 7.5 Hz), 1.68 (4H, p, J = 7.5 Hz), 1.78 (6H, s), 1.99 (6H, s), 2.14 (6H, s), 2.21 (6H, s), 2.33 (8H, t, J = 7.5 Hz), 3.66 (6H, s), 5.56 (2H, s), 6.74 (2H, s), 9.16 (2H, s), 12.31 (1H, s) ppm; ¹³C NMR (CDCl₃): δ = 9.0, 9.9, 10.3, 24.9, 25.3, 29.5, 29.9, 34.5, 51.9, 97.3, 113.4, 129.6, 129.7, 136.9, 138.2, 139.9, 140.0, 143.3, 154.8, 168.5, 171.4, 174.7 ppm.

Diethyl 2,5-diformyl-1H-pyrrole-3,4-dipropionate (8, C₁₆H₂₁NO₆)

In a 25 cm³ round-bottom flask were added 40 mg pyrrole **23** (0.15 mmol) and 8 cm³ CH₃OH. Trifluoroacetic acid (2 cm³) was added, and the mixture was stirred at room temperature overnight under nitrogen. Triethyl orthoformate (2 cm³) was added to the solution and stirred overnight under N₂. The solvents were removed (roto-vap), and the resulting oil was purified by flash chromatography (TLC silica gel deactivated with 10% H₂O using CH₂Cl₂-CH₃OH 99.5:0.5 (by vol) as eluent. This procedure gave **8** as a light pink solid. Yield: 32 mg (0.11 mmol, 73%); m.p.: 115–117 °C; IR (KBr): ν = 3437, 2955, 1734, 1637, 1439, 1375, 1202, 1174, 1049, 732 cm⁻¹; ¹H NMR (CDCl₃): δ = 2.63 (4H, t, J = 7.3 Hz), 3.09 (4H, t, J = 7.3 Hz), 3.66 (6H, s), 9.80 (1H, bs), 9.90 (2H, s) ppm; ¹³C NMR (CDCl₃): δ = 15.6, 32.6, 48.7, 128.2, 128.8, 169.3, 177.0 ppm.

Methyl 2-[(1,5-dihydro-3,4-dimethyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-1H-pyrrole-3-propanoate (9, C₁₆H₂₀N₂O₃)

Excess ethereal diazomethane was added to 100 mg dipyrinone **30** (0.36 mmol) suspended in 60 cm³ CH₃OH. The mixture was stirred for 1 h, after which the solvent was evaporated to dryness (roto-vap). The residue dissolved in 5 cm³ of CH₂Cl₂ and passed through a short column of silica gel (Woelm TLC grade F-DC 35/41, 10% water), eluting with CH₂Cl₂-CH₃OH 99:1 (by vol) to afford very pure methyl ester **9**. Yield: 95 mg (92%); m.p.: 177–178 °C (dec); IR (KBr): ν = 3447, 3366, 2919, 1737, 1672, 1637, 1443, 1402, 1349, 1273, 1169, 691, 485 cm⁻¹; ¹H NMR (CDCl₃): δ = 1.92 (3H, s), 2.07 (3H, s), 2.14 (3H, s), 2.50 (2H, t, J = 7.8 Hz), 2.90 (2H, t, J = 7.8 Hz), 3.65 (3H, s), 6.16 (1H, s), 6.82 (1H, s), 10.45 (1H, s), 11.09 (1H, s) ppm; ¹³C NMR (CDCl₃): δ = 8.7, 10.4, 10.5, 20.5, 36.0, 52.1, 101.4, 119.3, 121.9, 124.6, 124.8, 127.0, 130.6, 143.2, 174.0, 174.8 ppm; UV-Vis (CHCl₃): λ_{max} (ϵ) = 389 (26700) nm (M⁻¹ cm⁻¹); UV-Vis (CH₃OH): λ_{max} (ϵ) = 393 (30200) nm (M⁻¹ cm⁻¹); UV-Vis ((CH₃)₂SO): λ_{max} (ϵ) = 389 (28900) nm (M⁻¹ cm⁻¹).

Methyl 2-[(1,5-dihydro-3,4-dimethyl-5-oxo-2H-pyrrol-2-ylidene)methyl]-4-methyl-1H-pyrrole-3-hexanoate (11, C₁₉H₂₆N₂O₃)

Excess ethereal diazomethane was added to 50 mg dipyrinone **31** (0.16 mmol) suspended in 30 cm³ CH₃OH and allowed to proceed to dryness (roto-vap) as above to give pure methyl ester **11**. Yield: 50 mg (95%); m.p.: 164–165 °C; IR (KBr): $\nu = 3425, 3366, 2931, 1737, 1696, 1672, 1637, 1273, 1350, 756, 585$ cm⁻¹; ¹H NMR (CDCl₃): $\delta = 1.38$ (2H, p, $J = 7.3$ Hz), 1.52 (2H, p, $J = 7.3$ Hz), 1.67 (2H, p, $J = 7.3$ Hz), 1.93 (3H, s), 2.05 (3H, s), 2.11 (3H, s), 2.31 (2H, t, $J = 7.3$ Hz), 2.55 (2H, t, $J = 7.3$ Hz), 3.66 (3H, s), 6.10 (1H, s), 6.82 (1H, d, $J = 2$ Hz), 10.44 (1H, s), 11.12 (1H, s) ppm; ¹³C NMR (CDCl₃): $\delta = 8.7, 10.4, 10.6, 24.8, 25.3, 29.4, 31.5, 34.5, 51.9, 101.6, 119.4, 121.9, 124.5, 124.7, 129.4, 130.2, 142.9, 174.6, 174.7$ ppm; UV-Vis (CHCl₃): $\lambda_{\text{max}} (\epsilon) = 393$ (25800) nm (M⁻¹ cm⁻¹); UV-Vis (CH₃OH): $\lambda_{\text{max}} (\epsilon) = 397$ (28800) nm (M⁻¹ cm⁻¹); UV-Vis ((CH₃)₂SO): $\lambda_{\text{max}} (\epsilon) = 397$ (27000) nm (M⁻¹ cm⁻¹).

Diethyl 5-acetyl-4-oxooctanedioate (13, C₁₄H₂₂O₆)

In a 100 cm³ round-bottom flask equipped with reflux condenser and a heating mantle, 20 g ethyl 4,6-dioxoheptanoate (**12**, 0.108 mol), 8.06 g ethyl acrylate (0.081 mol), 2.35 g potassium fluoride (KF·2H₂O, 0.025 mol), and 10 cm³ absolute ethanol were combined, and the mixture was heated to 60 °C for 11 h. After cooling to room temperature, 30 cm³ H₂O was added, and the mixture was transferred to a separatory funnel. The organic compounds were extracted into CH₂Cl₂ (3 × 50 cm³), combined, washed with H₂O (2 × 50 cm³), and dried over anhyd. Na₂SO₄. The solvent was removed (roto-vap), and residual liquid was distilled as a clear liquid. Yield: 27.4 g (0.091 mol, 89%); b.p.: 146–148 °C (0.1 mbar); IR (neat): $\nu = 2984, 2939, 1732, 1703, 1376, 1184$ cm⁻¹; ¹H NMR (CDCl₃): $\delta = 1.11$ (3H, t, $J = 7.2$ Hz), 1.12 (3H, t, $J = 7.2$ Hz), 2.03 (2H, m), 2.08 (3H, s), 2.17 (2H, t, $J = 6.9$ Hz), 2.46 (2H, t, $J = 6$ Hz), 2.66 (2H, t, $J = 6$ Hz), 3.69 (1H, t, $J = 6.9$ Hz), 3.97 (2H, q, $J = 7.2$ Hz), 3.98 (2H, q, $J = 7.2$ Hz) ppm; ¹³C NMR (CDCl₃): $\delta = 13.9$ (q), 22.6 (t), 27.6 (t), 28.8 (q), 31.2 (t), 36.6 (t), 60.2 (t), 60.4 (t), 66.0 (d), 172.0 (s), 172.3 (s), 203.2 (s), 204.1 (s) ppm; GC-MS: $m/z = 286$ [M⁺14], 244, 241. It was used directly in the next step.

Diethyl 2-(ethoxycarbonyl)-5-methyl-1H-pyrrole-3,4-dipropanoate (14)

To a 250 cm³ two neck round-bottom flask equipped with a football stir bar, dropping funnel, and thermometer were added 14.3 g diethyl 5-acetyl-4-oxooctanedioate (**13**, 0.05 mol) and 50 cm³ glacial acetic acid. The solution was warmed to 80 °C using a heating mantle, then 12.8 g anhydrous sodium acetate (0.16 mol) and 10.9 g Zn dust (0.17 g-atom) were added with vigorous stirring. After heating the mixture to 115 °C, a solution of 9.4 g diethyl oximinomalonate (0.05 mol) in 15 cm³ glacial acetic acid and 5 cm³ H₂O was added drop-wise over 1 h. The temperature was maintained at 115 °C and stirred for 8.5 h. The hot liquid was then poured from the Zn onto 200 cm³ ice with vigorous stirring. On standing for 12 h, two layers formed. The upper pyrrole layer was extracted from the aqueous layer into CH₂Cl₂ (2 × 150 cm³) and dried over anhyd. Na₂SO₄. The solvent was removed (roto-vap) to give **14** as a viscous oil. Yield: 10.2 g (0.029 mol, 71%); b.p.: 155–160 °C (0.27 mbar) (Ref. [56] m.p.: 40–41 °C); ¹H NMR (CDCl₃): $\delta = 1.18$ (3H, t, $J = 7$ Hz), 1.20 (3H, t, $J = 7$ Hz), 1.29 (3H, t, $J = 7$ Hz), 2.17 (3H, s), 2.36 (2H, t, $J = 7.5$ Hz, 8.0 Hz), 2.48 (2H, t, $J = 7.6$

Hz, 8.6 Hz), 2.67 (2H, t, $J = 7.5$ Hz, 8.0 Hz), 2.94 (2H, t, $J = 7.6$ Hz, 8.6 Hz), 4.06 (2H, q, $J = 7$ Hz), 4.08 (2H, q, $J = 7$ Hz), 4.24 (2H, q, $J = 7$ Hz), 9.37 (1H, broad s) ppm; ^{13}C NMR (CDCl_3): $\delta = 11.2$ (q), 13.95 (q), 13.99 (q), 14.2 (q), 19.1 (t), 20.6 (t), 35.4 (t), 35.5 (t), 59.7 (t), 60.0 (t), 60.1 (t), 116.5 (s), 119.4 (s), 129.5 (s), 130.3 (s), 161.2 (s), 172.8 (s), 173.0 (s) ppm; GC-MS: $m/z = 353$ [M^+17], 308, 279, 234, 192, 146, 91.

2,5-Dicarboxy-1H-pyrrole-3,4-dipropanoic acid (**15**, $\text{C}_{12}\text{H}_{15}\text{NO}_8$)

Dry pyrrole **14** (7.0 g, 0.02 mol) was dissolved in 50 cm^3 dry THF and added to a 100 cm^3 round-bottom flask equipped with an addition funnel. The mixture was cooled to -20 $^\circ\text{C}$ in a dry ice-2-propanol bath, and 9.3 g freshly distilled SO_2Cl_2 (69 mmol) was added drop-wise with stirring at a constant temperature of -15 $^\circ\text{C}$. The addition took 10 min. The reaction mixture was then stirred at -10 $^\circ\text{C}$ for 1.5 h and then at 5 $^\circ\text{C}$ for 3.5 h. The mixture was then quenched with 5 cm^3 H_2O , added drop-wise and with the temperature kept below 7 $^\circ\text{C}$. After allowing the quenched reaction mixture to come to room temperature overnight, the THF and most of the H_2O were removed (roto-vap). The residue, when cooled to -30 $^\circ\text{C}$ afforded a white solid, which was collected by suction filtration and washed with CH_2Cl_2 to afford 2.8 g of a mixture of three pyrroles. These were immediately dissolved in 35 cm^3 95% ethanol and transferred to a 250 cm^3 round-bottom flask equipped with a reflux condenser and a heating mantle. Potassium hydroxide (1 M aq., 130 cm^3) was added all at once to the stirred solution, and the mixture was heated at reflux for 3.5 h. The ethanol and most of the H_2O were then removed (roto-vap), and the reaction was cooled to 5 $^\circ\text{C}$. Nitric acid (40 cm^3) was added dropwise, with the temperature kept below 7 $^\circ\text{C}$. The resulting white precipitate was collected and dried overnight in a dessicator at 0.07 mbar. Yield: 2.8 g (7.3 mmol, 40%); m.p.: 209 – 210 $^\circ\text{C}$ (dec); IR (KBr): $\nu = 3020, 1695, 1555, 1385, 1276$ cm^{-1} ; ^1H NMR ($(\text{CD}_3)_2\text{SO}$): $\delta = 2.33$ (4H, t, $J = 7.3$ Hz, 8.3 Hz), 2.82 (4H, t, $J = 7.3$ Hz, 8.3 Hz), 11.36 (1H, s), 12.42 (4H, bs) ppm; ^{13}C NMR ($(\text{CD}_3)_2\text{SO}$): $\delta = 19.7, 53.1, 122.1, 128.8, 161.6, 174.0$ ppm. The tetra-acid was used directly in the following step.

Diethyl 2,5-dicarboxy-1H-pyrrole-3,4-dipropanoate (**16**, $\text{C}_{16}\text{H}_{23}\text{NO}_8$)

In a 50 cm^3 round-bottom flask were added in sequence: 2 g **15** (6.7 mmol), 35 cm^3 absolute ethanol, and 3 cm^3 TFA, and the mixture was stirred at room temperature overnight under N_2 . Triethyl orthoformate (3 cm^3) was added to the solution and stirred overnight under N_2 . The solvents were removed (roto-vap) leaving **16** as a light pink solid. Yield: 2.4 g (6.7 mmol, 100%); m.p.: 179 – 180 $^\circ\text{C}$ (dec); IR (KBr): $\nu = 3419, 2987, 1700, 1558, 1469, 1384, 1263, 1180, 1044, 826$ cm^{-1} ; ^1H NMR ($(\text{CD}_3)_2\text{SO}$): $\delta = 1.11$ (6H, t, $J = 7.2$ Hz), 2.41 (4H t, $J = 7.8$ Hz), 2.85 (4H t, $J = 7.8$ Hz), 3.98 (4H, q, $J = 7.2$ Hz), 11.41 (1H, s), 12.65 (2H, bs) ppm; ^{13}C NMR ($(\text{CD}_3)_2\text{SO}$): $\delta = 14.1, 19.6, 34.9, 59.8, 122.2, 128.4, 161.5, 172.3$ ppm.

Diethyl 1H-pyrrole-3,4-dipropanoate (**17**, $\text{C}_{14}\text{H}_{21}\text{NO}_4$)

Pyrrole diacid **16** (1.85 g, 5.4 mmol), 3.5 g sodium acetate trihydrate, and 3.5 g potassium acetate were ground together in a mortar, and the intimate mixture was transferred with a spatula to a 25 cm^3 round-bottom flask equipped with a condenser, magnetic stirrer, and an inlet through which a nitrogen atmosphere could be maintained throughout the procedure. The reaction mixture was immersed in an oil bath at 105 $^\circ\text{C}$ and the temperature was rapidly

increased. At 100 °C (bath) liquefaction occurred. Stirring was started and at 115 °C decarboxylation began. The bath was maintained at 135–140 °C until the evolution of carbon dioxide ceased (ca. 30 min). The reaction mixture was then cooled to room temperature and partitioned between H₂O and CH₂Cl₂. The H₂O layer was washed with three portions of CH₂Cl₂ (3 × 15 cm³), which were then combined and washed three times with 5% aq. NaHCO₃ (3 × 25 cm³), and dried over anhyd. Na₂SO₄. After filtration to remove Na₂SO₄ the solvent was removed (roto-vap) leaving the desired diester **17** as an extremely unstable colorless oil, which was used immediately in the next step. Yield: 0.86 g (62%); ¹H NMR (CDCl₃): δ = 1.25 (6H, t, *J* = 7.2 Hz), 2.56 (4H, dd, *J* = 8.5 Hz, 9.3 Hz), 2.77 (4H, dd, *J* = 8.5 Hz, 9.3 Hz), 4.13 (4H, q, *J* = 7.2 Hz), 6.52 (2H, s), 7.97 (1H, bs) ppm; ¹³C NMR (CDCl₃): δ = 13.8, 20.2, 34.7, 59.9, 114.8, 120.5, 173.2 ppm; GC-MSD: *m/z* = 267 (M⁺15), 222, 194, 180, 148, 106, 94.

Diethyl 2-formyl-1H-pyrrole-3,4-dipropanoate (**18**, C₁₁H₁₃NO₅)

Pyrrole diester **17** (0.80 g, 3.0 mmol) was dissolved in 35 cm³ anhyd. ethyl ether in a 50 cm³ round-bottom flask, 0.5 cm³ *N,N*-dimethylformamide was added, and the flask was submerged in an ice bath and cooled to 5 °C. Phosphorous oxychloride (0.5 cm³) was then added drop-wise with stirring at a constant temperature of 5 °C. The mixture was allowed to come to room temperature while stirring overnight under a blanket of N₂. The ethyl ether was removed (roto-vap), and 3 cm³ water was added. The mixture was cooled to 5 °C in an ice bath, and 2.3 g NaOH in 8 cm³ H₂O was added carefully. The solution was then heated to just below boiling for 20 min and then cooled to 5 °C. Concentrated HCl was added slowly until pH 3 was reached. The solution was then stirred for 1 h while a precipitate formed. The solid was collected by suction filtration and washed with 2 cm³ H₂O. The beige solid was recrystallized from a minimal amount of warm CH₃OH and cooled to give **18** as a white solid. Yield: 0.4 g (1.7 mmol, 56%); m.p.: 156–157 °C; IR (KBr): ν = 3439, 1711, 1623, 1428, 1369, 1253, 1183, 823 cm⁻¹; ¹H NMR (CD₃OD): δ = 2.54 (2H, t, *J* = 7.5 Hz), 2.55 (2H, t, *J* = 7.2 Hz), 2.75 (2H, t, *J* = 7.2 Hz), 3.03 (2H, t, *J* = 7.5 Hz), 6.96 (1H, s), 9.52 (1H, s) ppm; ¹³C NMR (CD₃OD): δ = 18.4, 19.0, 34.0, 34.9, 104.0, 123.5, 124.6, 128.8, 174.6, 178.05, 178.09 ppm.

2-[2-Cyano-2-(ethoxycarbonyl)vinyl]-1H-pyrrole-3,4-dipropanoic acid (**19**, C₁₈H₁₈N₂O₆)

Pyrrole **18** (50 mg, 0.21 mmol) and 40 mg ethyl cyanoacetate (3 drops) were combined in a 2 cm³ round-bottom flask. Absolute ethanol (1 cm³) was added, and the mixture was stirred for 15 min while the solids dissolved. Diethyl amine (2 drops) was added, and the mixture was heated at reflux for 4 h. The mixture was then cooled to room temperature, and the solvent was removed (roto-vap). The salts were taken up in a minimal amount of warm H₂O and cooled to 5 °C in an ice bath. Upon adding one drop of concentrated HCl, the desired pyrrole precipitated and was collected by suction filtration to afford a non-optimized yield of the desired protected aldehyde. Yield: 33 mg (0.10 mmol, 47%); m.p.: 195–196 °C (dec); IR (KBr): ν = 3425, 1718, 1655, 1592, 1546, 1252, 850 cm⁻¹; ¹H NMR ((CD₃)₂SO): δ = 1.23 (3H, t, *J* = 7.2 Hz), 2.34 (2H, t, *J* = 7.2 Hz), 2.46 (2H, t, *J* = 7.5 Hz), 2.59 (2H, t, *J* = 7.2 Hz), 2.78 (2H, t, *J* = 7.5 Hz), 4.20 (2H, q, *J* = 7.2 Hz), 7.19 (1H, d, *J* = 3.3 Hz), 7.95 (1H, s), 10.91 (1H, s), 12.16 (2H, bs) ppm; ¹³C NMR ((CD₃)₂SO): δ = 14.2, 19.3, 19.6, 34.2, 35.5, 61.4, 88.2, 117.6, 123.3, 124.8, 129.8, 136.0, 139.6, 163.6, 173.4, 173.9 ppm.

Diethyl 2-[2-cyano-2-(ethoxycarbonyl)vinyl]-1H-pyrrole-3,4-dipropionate (20, C₂₀H₂₆N₂O₆)

In a 5 cm³ round-bottom flask, 15 mg pyrrole **19** (0.045 mmol) was dissolved in 2 cm³ absolute ethanol with 6 drops of TFA and stirred at room temperature overnight under N₂. Triethyl orthoformate (6 drops) was added to the solution, which was stirred an additional 20 h under N₂. The solvents were removed (roto-vap), and the resulting beige product was collected. Yield: 17 mg (98%); IR (film): $\nu = 3397, 2984, 2212, 1731, 1590, 1370, 1230, 1177, 1094, 1017\text{ cm}^{-1}$; ¹H NMR (CDCl₃): $\delta = 1.23$ (3H, t, $J = 7.2$ Hz), 1.24 (3H, t, $J = 7.2$ Hz), 1.36 (3H, t, $J = 7.2$ Hz), 2.50 (2H, t, $J = 7.5$ Hz), 2.58 (2H, t, $J = 7.5$ Hz), 2.79 (2H, t, $J = 7.5$ Hz), 2.95 (2H, t, $J = 7.5$ Hz), 4.11 (2H, q, $J = 7.2$ Hz), 4.13 (2H, q, $J = 7.2$ Hz), 4.33 (2H, q, $J = 7.2$ Hz), 7.03 (1H, d, $J = 3$ Hz), 8.03 (1H s), 9.81 (1H, bs) ppm; ¹³C NMR (CDCl₃): $\delta = 13.75, 13.80, 13.9, 19.0, 19.5, 34.2, 35.5, 60.3, 60.4, 61.6, 89.6, 118.8, 123.9, 124.5, 126.4, 135.1, 139.0, 163.3, 172.3, 176.9$ ppm; GC-MSD: $m/z = 390$ (M⁺10), 317, 303, 256, 184, 155, 130.

Diethyl 2-formyl-1H-pyrrole-3,4-dipropionate (21, C₁₆H₂₁NO₅)

In a 50 cm³ round-bottom flask, 0.59 g pyrrole **17** (2.2 mmol) was dissolved in 30 cm³ anhyd. ethyl ether, then 1.0 cm³ dimethylformamide was added, and the flask was submerged in an ice bath and cooled to 5 °C. Phosphorous oxychloride (1.5 cm³) was added drop-wise with stirring at a constant temperature of 5 °C. The mixture was allowed to come to room temperature while stirring overnight under a blanket of nitrogen. The ethyl ether was then removed (roto-vap), and the resulting oil was re-dissolved in 10 cm³ 1,2-dichloroethane. Sodium acetate trihydrate (7.5 g in 35 cm³ H₂O) was added, and the mixture was heated at reflux for 20 min, then cooled to room temperature. The reaction mixture was extracted with CH₂Cl₂ (3 × 20 cm³), dried over anhyd. Na₂SO₄, and the solvents were removed, giving **21** as a light brown oil. Yield: 0.53 g (1.8 mmol, 82%); b.p.: 72–75 °C; IR (film): $\nu = 3265, 2983, 2934, 1732, 1652, 1558, 1507, 1374, 1256, 1179, 1097, 1036, 798\text{ cm}^{-1}$; ¹H NMR (CDCl₃): $\delta = 1.20$ (3H, t, $J = 7.2$ Hz), 1.22 (3H, t, $J = 7.2$ Hz), 2.55 (4H, t, $J = 7.5$ Hz), 2.75 (2H, t, $J = 7.5$ Hz), 3.03 (2H, t, $J = 7.5$ Hz), 4.09 (2H, q, $J = 7.2$ Hz), 4.11 (2H, q, $J = 7.2$ Hz), 6.90 (1H, s), 9.58 (1H, s), 10.04 (1H, bs) ppm; ¹³C NMR (CDCl₃): $\delta = 14.0, 18.8, 19.6, 34.7, 36.0, 60.3, 60.4, 123.7, 124.4, 129.2, 132.6, 172.2, 172.6, 177.7$ ppm; GC-MSD: $m/z = 295$ (M⁺10), 267, 250, 220, 175, 148, 134, 106.

Diethyl 2-[2-cyano-2-(ethoxycarbonyl)vinyl]-5-formyl-1H-pyrrole-3,4-dipropionate (22, C₂₁H₂₆N₂O₇)

In a 100 cm³ round-bottom flask, 1.0 cm³ *N,N*-dimethylformamide was cooled to 0 °C, then 1.0 cm³ phosphorous oxychloride was added slowly over a period of 15 min. The resulting paste was warmed to room temperature and stirred for 10 min. Dry 1,2-dichloroethane (5 cm³) was added, followed by 0.53 g pyrrole **20** (1.36 mmol) dissolved in 10 cm³ dry 1,2-dichloroethane. The flask was equipped with a reflux condenser, and the mixture was heated at reflux for 20 min. The solution was then cooled to room temperature, 7.5 g sodium acetate trihydrate in 30 cm³ H₂O was added, and the mixture was heated to reflux for an additional 15 min, then cooled to room temperature. The reaction was extracted with CH₂Cl₂ (3 × 20 cm³) and dried over anhyd. Na₂SO₄. The solvent was removed (roto-vap) and the resulting compound was purified by flash chromatography (TLC silica gel deactivated with 10%

H₂O) using CH₂Cl₂-CH₃OH 99.5:0.5 (by vol) as eluent to give pyrrole **22** as a light brown oil. Yield: 0.46 g (1.11 mmol, 81%); m.p.: 55–56 °C; IR (film): ν = 3409, 2984, 2215, 1730, 1660, 1590, 1448, 1372, 1230, 1177, 1094, 1017 cm⁻¹; ¹H NMR (CDCl₃): δ = 1.19 (3H, t, *J* = 7.2 Hz), 1.20 (3H, t, *J* = 7.2 Hz), 1.35 (3H, t, *J* = 7.2 Hz), 2.50 (2H, t, *J* = 7.5 Hz), 2.58 (2H, t, *J* = 7.5 Hz), 2.95 (2H, t, *J* = 7.5 Hz), 3.07 (2H, t, *J* = 7.5 Hz), 4.08 (4H, q, *J* = 7.2 Hz), 4.34 (2H, q, *J* = 7.2 Hz), 8.10 (1H, s), 9.88 (1H, s), 10.22 (1H, bs) ppm; ¹³C NMR (CDCl₃): δ = 14.0, 18.4, 18.7, 35.4, 35.6, 60.6, 60.6, 62.5, 97.6, 117.1, 127.4, 130.7, 133.1, 134.6, 139.0, 162.0, 171.6, 171.8, 179.1 ppm; GC-MSD: *m/z* = 418 (M⁺15), 343, 299, 298, 271, 211, 155, 142, 55.

2,5-Diformyl-1H-pyrrole-3,4-dipropanoic acid (**23**, C₁₂H₁₃NO₆)

In a 50 cm³ round-bottom flask were added 0.10 g pyrrole **22** (0.24 mmol) and 1.5 cm³ ethanol. The mixture was stirred until the oil was dissolved (ca. 15 min). Potassium hydroxide (2 cm³ of a 50% aq. solution) was then added, and a condenser was attached to the flask. The mixture was heated at reflux for 3 h, allowed to cool to room temperature, chilled in an ice bath, and then acidified carefully with 6 N H₂SO₄ to pH 3. The resulting precipitate was dissolved in 15 cm³ H₂O and extracted thoroughly with diethyl ether (5 × 25 cm³). The organic phases were combined, dried over anhyd. MgSO₄, filtered, and the solvent was removed (roto-vap) to afford **23**. Yield: 41 mg (0.15 mmol, 67%); ¹H NMR (CD₃OD): δ = 2.57 (2H, t, *J* = 7.3 Hz), 3.07 (2H, t, *J* = 7.3 Hz) ppm. This very unstable compound was used immediately to form **8**.

Ethyl 5-(ethoxycarbonyl)-2-formyl-4-methyl-1H-pyrrole-3-propanoate (**26**)

In a 500 cm³ round-bottom flask 2.0 g ethyl 5-(ethoxycarbonyl)-2,4-dimethyl-1H-pyrrole-3-propanoate [45] (7.5 mmol), 60 cm³ THF, 120 cm³ glacial acetic acid, and 100 cm³ H₂O were combined. The mixture was placed into an ice bath and stirred magnetically for 30 min. Cerium(IV) ammonium nitrate (16.6 g) was added all at once, and the mixture was stirred at 5 °C for 1 h. The mixture was allowed to warm to room temperature, transferred to a separatory funnel, and 500 cm³ water was added. The solution was extracted with CH₂Cl₂ (3 × 100 cm³), and the organic phases were then combined, washed with aq. NaHCO₃ (5%, 4 × 100 cm³), and dried over anhyd. Na₂SO₄. Removal of the solvent (roto-vap) gave **26** as a light beige solid. Yield: 2.0 g (7.1 mmol, 95%); m.p.: 61–62 °C (Lit. [45] m.p.: 62–63 °C); ¹H NMR (CDCl₃): δ = 1.14 (3H, t, *J* = 7.5 Hz), 1.29 (3H, t, *J* = 7.5 Hz), 2.22 (3H, s), 2.49 (2H, t, *J* = 7.3 Hz), 2.98 (2H, t, *J* = 7.3 Hz), 4.01 (2H, q, *J* = 7.5 Hz), 4.26 (2H, q, *J* = 7.5 Hz), 9.74 (1H, s), 9.98 (1H, bs) ppm; GC-MSD: *m/z* = 281 (M⁺4), 253, 236, 207, 194, 179, 166, 162, 148, 133, 120, 106.

Ethyl 5-(ethoxycarbonyl)-2-formyl-4-methyl-1H-pyrrole-3-hexanoate (**27**, C₁₇H₂₅NO₅)

In a 500 cm³ round-bottom flask, 2.0 g pyrrole **25** [41] (6.5 mmol), 60 cm³ THF, 120 cm³ glacial acetic acid, and 100 cm³ H₂O were combined. The mixture was placed into an ice bath and stirred magnetically for 30 min. Cerium(IV) ammonium nitrate (16.6 g) was added all at once, and the mixture was stirred at 5 °C for 1 h. The mixture was allowed to warm to room temperature, transferred to a separatory funnel, and 500 cm³ H₂O was added. The solution was extracted with CH₂Cl₂ (3 × 100 cm³), and the organic phases were then

combined, washed with aq. NaHCO_3 (5%, $4 \times 100 \text{ cm}^3$), dried over anhyd. Na_2SO_4 , then the solvent was removed (roto-vap). This procedure afforded **27** that was recrystallized from ethanol. Yield: 2.0 g (6.2 mmol, 96%); m.p.: 52–52.5 °C; IR (KBr): $\nu = 3448, 2935, 1731, 1719, 1664, 1465, 1384, 1264, 1100, 1019 \text{ cm}^{-1}$; ^1H NMR (CDCl_3): $\delta = 1.21$ (3H, t, $J = 7.3$ Hz), 1.35 (3H, t, $J = 7.3$ Hz), 1.37 (2H, p, $J = 7.5$ Hz), 1.54 (2H, p, $J = 7.5$ Hz), 1.63 (2H, p, $J = 7.5$ Hz), 2.25 (3H, s), 2.26 (2H, t, $J = 7.5$ Hz), 2.69 (2H, t, $J = 7.5$ Hz), 3.97 (2H, q, $J = 7.3$ Hz), 4.21 (2H, q, $J = 7.3$ Hz), 9.63 (1H, bs), 9.72 (1H, s) ppm; ^{13}C NMR: $\delta = 6.6, 11.0, 11.2, 20.2, 21.6, 25.6, 28.0, 31.0, 57.0, 57.7, 121.6, 123.2, 127.0, 131.5, 157.9, 170.4, 176.4$ ppm; GC-MSD: $m/z = 323$ (M^+), 294, 278, 248, 204, 166, 162, 148, 120, 92, 65.

2-[(1,5-Dihydro-3,4-dimethyl-5-oxo-2H-pyrrole-2-ylidene)methyl]-5-carboxy-4-methyl-1H-pyrrole-3-propanoic acid (28, $\text{C}_{16}\text{H}_{18}\text{N}_2\text{O}_5$)

Pyrrole **26** (1.0 g, 3.6 mmol), 0.5 g 3,4-dimethyl-3-pyrrolin-2-one (4.5 mmol), and 10 cm^3 95% ethanol were combined in a 25 cm^3 round-bottom flask and the mixture was stirred at room temperature until the solids dissolved. Then aqueous KOH (1.0 cm^3 of a 4.0 M solution) was added drop-wise. The mixture was blanketed with N_2 and stirred under nitrogen in the dark for 48 h. The mixture was then cooled to 5 °C in an ice bath and glacial acetic acid was added until a pH of 5 was obtained. The solution was allowed to stir for an additional 5 min while the yellow precipitate formed. The precipitate was collected by filtration, washed with an excess of ice-cold water, and dried under vacuum (2 mbar). This procedure afforded **28**. It was recrystallized from $(\text{CH}_3)_2\text{SO}$ and H_2O . Yield: 0.55 g (1.7 mmol, 48%); m.p.: 239–240 °C; IR (KBr): $\nu = 3366, 3190, 2919, 1678, 1472, 1402, 1267, 1534, 938 \text{ cm}^{-1}$; ^1H NMR ($(\text{CD}_3)_2\text{SO}$): $\delta = 1.75$ (3H, s), 2.04 (3H, s), 2.18 (3H, s), 2.30 (2H, t, $J = 7.5$ Hz), 2.72 (2H, t, $J = 7.5$ Hz), 5.98 (1H, s), 10.52 (1H, s), 10.96 (1H, s), 12.3 (2H, bs) ppm; ^{13}C NMR ($(\text{CD}_3)_2\text{SO}$): $\delta = 5.7, 6.9, 7.5, 16.7, 32.6, 93.3, 119.2, 123.1, 123.7, 123.8, 124.8, 131.7, 139.5, 159.6, 170.1, 171.2$ ppm; UV-Vis (CH_3OH): $\lambda_{\text{max}} (\epsilon) = 381$ (27400), 398 (24000) nm ($\text{M}^{-1} \text{ cm}^{-1}$); UV-Vis ($(\text{CH}_3)_2\text{SO}$): $\lambda_{\text{max}} (\epsilon) = 382$ (29900), 402 (27000) nm ($\text{M}^{-1} \text{ cm}^{-1}$).

2-[(1,5-Dihydro-3,4-dimethyl-5-oxo-2H-pyrrole-2-ylidene)methyl]-5-carboxy-4-methyl-1H-pyrrole-3-hexanoic acid (29, $\text{C}_{19}\text{H}_{24}\text{N}_2\text{O}_5$)

Pyrrole **27** (1.0 g, 3.6 mmol), 0.43 g 3,4-dimethyl-3-pyrrolin-2-one (3.9 mmol), and 15 cm^3 95% ethanol were combined in a 25 cm^3 round-bottom flask. The mixture was stirred magnetically at room temperature until the solids dissolved and reacted as in the preparation of **28**. The precipitate formed during work-up was collected by centrifugation, washed twice with ice-cold H_2O (separation by centrifugation) and dried under vacuum (2 mbar). This procedure afforded dipyrinone **29**, which was recrystallized from an excess of warm CH_3OH . Yield: 0.54 g (1.5 mmol, 50%); m.p.: 214 °C; IR (KBr): $\nu = 3376, 3187, 2934, 1677, 1474, 1406, 1263, 1179, 946 \text{ cm}^{-1}$; ^1H NMR ($(\text{CD}_3)_2\text{SO}$): $\delta = 1.24$ (2H, p, $J = 7.3$ Hz), 1.35 (2H, p, $J = 7.3$ Hz), 1.48 (2H, p, $J = 7.3$ Hz), 1.75 (3H, s), 2.02 (3H, s), 2.14 (2H, t, $J = 7.3$ Hz), 2.16 (3H, s), 2.44 (2H, t, $J = 7.3$ Hz), 5.85 (1H, s), 10.53 (1H, s), 10.94 (1H, s), 11.48 (2H, bs) ppm; ^{13}C NMR ($(\text{CD}_3)_2\text{SO}$): $\delta = 5.7, 6.9, 7.6, 20.7, 21.7, 25.6, 27.8, 31.1, 93.2, 119.1, 123.0, 123.8, 124.6, 125.2, 131.5, 139.2, 159.6, 170.1, 171.7$ ppm; UV-Vis

(CH₃OH): λ_{max} (ϵ) = 382 (28100), 401 (25000) nm (M⁻¹ cm⁻¹); UV-Vis ((CH₃)₂SO): λ_{max} (ϵ) = 385 (30800), 405 (28800) nm (M⁻¹ cm⁻¹).

2-[(1,5-Dihydro-3,4-dimethyl-5-oxo-2H-pyrrole-2-ylidene)methyl]-4-methyl-1H-pyrrole-3-propanoic acid (30, C₁₅H₁₈N₂O₃)

Dipyrrinone **28** (0.12 g, 0.37 mmol), 0.55 g sodium acetate trihydrate, and 0.6 g potassium acetate were ground together in a mortar and the intimate mixture was transferred with a spatula to a 25 cm³ round-bottom flask equipped with a condenser, magnetic stirrer, and inlet through which a nitrogen atmosphere could be maintained throughout the procedure. The reaction mixture was immersed in an oil bath at 115 °C and the temperature was rapidly increased. At 117 °C (bath) liquefaction occurred. Stirring was started and at 120 °C decarboxylation began. The bath was maintained at 135–140 °C until the evolution of CO₂ ceased (ca. 25 min). The reaction mixture was then cooled to room temperature and suspended in 30 cm³ H₂O. Concentrated HCl was added (7 drops to pH 3) and the resulting yellow precipitate was collected by centrifugation, washed twice with ice-cold H₂O (separation by centrifugation) and dried under vacuum (2 mbar). This procedure yielded dipyrinone **30** as a bright yellow solid. Yield: 85 mg (0.31 mmol, 85%); m.p.: 220–222 °C (dec); IR (KBr): ν = 3448, 3354, 2919, 1690, 1637, 1402, 1273, 1249, 1173, 944, 697, 497 cm⁻¹; ¹H NMR ((CD₃)₂SO): δ = 1.75 (3H, s), 1.94 (3H, s), 2.04 (3H, s), 2.30 (2H, t, J = 7.5 Hz), 2.71 (2H, t, J = 7.5 Hz), 6.02 (1H, s), 6.71 (1H, s), 9.71 (1H, s), 10.47 (1H, s), 12.07 (1H, s) ppm; ¹³C NMR ((CD₃)₂SO): δ = 8.8, 10.0, 10.3, 19.9, 35.9, 98.3, 118.3, 120.6, 124.1, 124.7, 125.7, 130.5, 142.2, 172.4, 174.4 ppm; UV-Vis (CHCl₃): λ_{max} (ϵ) = 385 (25100) nm (M⁻¹ cm⁻¹); UV-Vis (CH₃OH): λ_{max} (ϵ) = 394 (30800) nm (M⁻¹ cm⁻¹); UV-Vis ((CH₃)₂SO): λ_{max} (ϵ) = 389 (29400) nm (M⁻¹ cm⁻¹).

2-[(1,5-Dihydro-3,4-dimethyl-5-oxo-2H-pyrrole-2-ylidene)methyl]-4-methyl-1H-pyrrole-3-hexanoic acid (31, C₁₈H₂₄N₂O₃)

Dipyrrinone **29** (100 mg, 0.28 mmol), 0.42 g sodium acetate trihydrate, and 0.45 g potassium acetate were ground together in a mortar and the intimate mixture was transferred with a spatula to a 25 cm³ round-bottom flask equipped with a condenser, magnetic stirrer, and an inlet through which a nitrogen atmosphere could be maintained throughout the procedure. The reaction was treated as in the preparation of **30** above to afford pure dipyrinone **31** as a bright yellow solid. Yield: 82 mg (0.26 mmol, 95%); m.p.: 281 °C (dec); IR (KBr): ν = 3436, 2931, 2367, 1672, 1637, 1402, 1173, 944, 497 cm⁻¹; ¹H NMR ((CD₃)₂SO): δ = 1.27 (2H, p, J = 7.3 Hz), 1.37 (2H, p, J = 7.3 Hz), 1.48 (2H, p, J = 7.3 Hz), 1.73 (3H, s), 1.91 (3H, s), 2.01 (3H, s), 2.14 (2H, t, J = 7.3 Hz), 2.42 (2H, t, J = 7.3 Hz), 5.88 (1H, s), 6.69 (1H, s), 9.70 (1H, s), 10.43 (1H, s), 11.93 (1H, bs) ppm; ¹³C NMR ((CD₃)₂SO): δ = 9.3, 10.5, 10.9, 24.5, 25.3, 29.2, 31.6, 34.7, 98.6, 118.7, 121.0, 124.5, 125.1, 127.8, 130.7, 142.5, 172.8, 175.4 ppm; UV-Vis (CHCl₃): λ_{max} (ϵ) = 399 (29400) nm (M⁻¹ cm⁻¹); UV-Vis (CH₃OH): λ_{max} (ϵ) = 396 (28200) nm (M⁻¹ cm⁻¹); UV-Vis ((CH₃)₂SO): λ_{max} (ϵ) = 398 (30000) nm (M⁻¹ cm⁻¹).

Acknowledgments

We thank the U.S. National Institutes of Health (R01 HD 17779) for support.

References

1. Bonnett R, Davies JE, Hursthouse MB, Sheldrick GM. *Proc Royal Soc Chem B*. 1978; 202:249.
2. Mugnoli A, Manitto P, Monti D. *Nature*. 1978; 273:568. [PubMed: 661967]
3. LeBas G, Allegret A, Mauguen Y, DeRango C, Bailly M. *Acta Crystallogr B*. 1980; 36:3007.
4. Chowdhury, JR.; Wolkoff, AN.; Chowdhury, NR.; Arias, IM. Hereditary jaundice and disorders of bilirubin metabolism. In: Scriver, CF.; Beaudet, AL.; Sly, WS.; Valle, D., editors. *The Metabolic Basis of Inherited Disease*. Vol. 125. McGraw-Hill; New York: 2000. p. 3063
5. Navon G, Frank S, Kaplan D. *J Chem Soc Perkin*. 1984; 2:1145.
6. Nogales D, Lightner DA. *J Biol Chem*. 1995; 270:73. [PubMed: 7814422]
7. Dörner T, Knipp B, Lightner DA. *Tetrahedron*. 1997; 53:2697.
8. Boiadjev SE, Anstine DT, Lightner DA. *J Am Chem Soc*. 1995; 117:8727.
9. Boiadjev SE, Anstine DT, Maverick E, Lightner DA. *Tetrahedron: Asymmetry*. 1995; 6:2253.
10. Tipton AK, Lightner DA. *Monatsh Chem*. 1999; 130:425.
11. Chen Q, Lightner DA. *J Org Chem*. 1998; 63:2665. [PubMed: 11672135]
12. Huggins MT, Lightner DA. *Tetrahedron*. 2000; 56:1797.
13. Huggins MT, Salzameda NT, Lightner DA. *Supramol Chem*. 2011; 23:226.
14. Nogales DF, Ma J-S, Lightner DA. *Tetrahedron*. 1993; 49:2361.
15. Huggins, MT.; Boidajiev, SE. Molecular recognition with dipyrinones and pyrrole-based derivatives. In: Atwood, JL.; Steed, JW., editors. *Encyclopedia of Supramolecular Chemistry*. Vol. 1. Taylor & Francis; London: 2010. p. 1
16. Dey SK, Lightner DA. *Monatsh Chem*. 2010; 141:101.
17. Nikitin EN, Lightner DA. *Monatsh Chem*. 2009; 140:97.
18. Boiadjev SE, Lightner DA. *Tetrahedron: Asymmetry*. 2004; 15:3301.
19. Ghosh B, Catalano VJ, Lightner DA. *Monatsh Chem*. 2004; 135:1305.
20. Tu B, Ghosh B, Lightner DA. *Tetrahedron*. 2004; 60:9017.
21. Ghosh B, Lightner DA, McDonagh AF. *Monatsh Chem*. 2004; 135:1189.
22. Ghosh B, Lightner DA. *J Heterocycl Chem*. 2003; 40:1113.
23. Huggins MT, Lightner DA. *J Org Chem*. 2000; 65:6001. [PubMed: 10987933]
24. Huggins MT, Lightner DA. *Tetrahedron*. 2001; 57:2279.
25. Huggins MT, Lightner DA. *J Org Chem*. 2001; 66:8402. [PubMed: 11735518]
26. Salzameda NT, Lightner DA. *Monatsh Chem*. 2007; 138:237.
27. Tu B, Ghosh B, Lightner DA. *Monatsh Chem*. 2004; 135:519.
28. Tu B, Ghosh B, Lightner DA. *J Org Chem*. 2003; 68:8950. [PubMed: 14604367]
29. Pfeiffer WP, Lightner DA. *Tetrahedron Lett*. 1994; 35:9673.
30. Nogales D, Anstine DT, Lightner DA. *Tetrahedron*. 1994; 50:8579.
31. Tipton AK, Lightner DA. *Monatsh Chem*. 1999; 130:425.
32. Chen Q, Lightner DA. *J Org Chem*. 1998; 63:2665. [PubMed: 11672135]
33. Huggins MT, Lightner DA. *Tetrahedron*. 2000; 56:1797.
34. Falk H, Flödl H. *Monatsh Chem*. 1985; 116:1177.
35. Montforts FP, Schwartz UM. *Liebig's Ann Chem*. 1985:1228.
36. Paine JB III, Woodward RB, Dolphin D. *J Org Chem*. 1976; 41:2826.
37. Byun YS, Lightner DA. *J Heterocycl Chem*. 1991; 28:1683.
38. Silverstein, RM.; Ryskiewicz, EE.; Willard, C. *Organic Syntheses*, coll. Rabjohn, N., editor. Vol. 4. Wiley; New York: 1963. p. 831
39. Riegel B, Lilienfeld W. *J Am Chem Soc*. 1945; 67:1273.
40. Paine JB III, Dolphin D. *Can J Chem*. 1976; 54:411.
41. Shrout DP, Lightner DA. *Synth Comm*. 1990; 20:2075.
42. Xie M, Lightner DA. *Tetrahedron*. 1993; 49:2185.

43. Woodward RB, Ayer WA, Beaton JM, Bickelhaupt F, Bonnett R, Buchschaler P, Closs GC, Dutler H, Hannah J, Hauck FP, Ito S, Langemann A, LeGoff E, Leimgruber W, Lwowski W, Sauer J, Valenta Z, Volz H. *Tetrahedron*. 1990; 46:7599.
44. Huggins MT, Lightner DA. *Monatsh Chem*. 2001; 132:203.
45. Puzicha G, Shrout DP, Lightner DA. *J Heterocycl Chem*. 1990; 27:2117.
46. Thyran T, Lightner DA. *Tetrahedron Lett*. 1995; 36:4345.
47. Falk H, Flödl H. *Monatsh Chem*. 1986; 117:57.
48. McDonagh AF, Palma LA, Trull FR, Lightner DA. *J Am Chem Soc*. 1982; 104:6865.
49. Xie M, Holmes DL, Lightner DA. *Tetrahedron*. 1993; 49:9235.
50. Nogales, DF. PhD Thesis. University of Nevada; Reno: 1993. Hydrogen bonding and solution conformation of bilirubin and its analogs.
51. Lightner DA, Gawro ski JK, Wijekoon WMD. *J Am Chem Soc*. 1987; 109:6354.
52. Lightner DA, Wijekoon WMD, Zhang MH. *J Biol Chem*. 1988; 263:16669. [PubMed: 3182807]
53. Lightner DA, Reisinger M, Landen GL. *J Biol Chem*. 1986; 261:6034. [PubMed: 3700382]
54. Rebek J Jr. *Angew Chem Int Ed*. 1990; 29:245.
55. Diederich F, Ferguson SB. *Angew Chem Int Ed*. 1986; 25:1127.
56. Chan DD, Clezy PS, Henderson RW, Pham H-P, Ravi BN. *Aust J Chem*. 1983; 36:1639.

Fig. 1.

(A) (left) Linear representation of bilirubin indicating which carboxylic acid is available to engage in hydrogen bonding to a given dipyrinone, resulting in (right) an intramolecularly hydrogen-bonded bilirubin in a ridge-tile conformation. (B) and (C) Centrally expanded analogs of bilirubin. (D) Intramolecularly hydrogen-bonded dipyrinones **D-1** and **D-2** and 3,18-desvinyl-3,18-mesobilirubin-IX α (**D-3**). (E) The target pentapyrroles **1**, **2**, and **3** of this work. Their corresponding dimethyl esters (not shown) are designated **1e**, **2e**, and **3e**, respectively

Fig. 2.
Interconverting intramolecularly hydrogen-bonded isoenergetic enantiomeric conformers of pentapyrrole **1** in a pseudo-ridge-tile form

Fig. 3.

(A) Diagrammatic interconversion summary of pentapyrrole **1** stable conformers. (B) line drawing depiction of the conformation of pentapyrrole **1** in a “U”-shape. (C) Ball and Stick representation of intramolecular hydrogen-bonded global minimum conformation of pentapyrrole **1**

Fig. 4.

(A) Pentapyrrole **1** cleft dimensions. (B) Ball and Stick representation of complexation between 5-fluorouracil and pentapyrrole **1** looking into the cleft from the propionic acid groups

Scheme 1.

Scheme 2.

a: $\text{CH}_3\text{CH}_2\text{OH}$ /reflux, then SOCl_2 ; b: $\text{Mg}(\text{OCH}_2\text{CH}_3)_2$ + *t*-butyl acetoacetate/cat. *p*-TsOH/ , then at 40 mbar; c: ethyl acrylate/KF/ $\text{CH}_3\text{CH}_2\text{OH}$; d: diethyl oximinomalonate/Zn/HOAc/NaOAc/reflux; e: SO_2Cl_2 , then KOH; f: $\text{HC}(\text{OCH}_2\text{CH}_3)_3/\text{CF}_3\text{CO}_2\text{H}$; g: KOAc/NaOAc/ ; h: $\text{POCl}_3/\text{DMF}/(\text{CH}_3\text{CH}_2)_2\text{O}$, then NaOH/reflux; i: $\text{NCCH}_2\text{CO}_2\text{CH}_2\text{CH}_3/(\text{CH}_3\text{CH}_2)_2\text{NH}/\text{CH}_3\text{CH}_2\text{OH}$ /reflux; j: $\text{POCl}_3/\text{DMF}/(\text{CH}_3\text{CH}_2)_2\text{O}$, then $\text{ClCH}_2\text{CH}_2\text{Cl}/\text{NaOAc}/\text{H}_2\text{O}$ /reflux; k: $\text{POCl}_3/\text{DMF}/\text{ClCH}_2\text{CH}_2\text{Cl}$, then NaOAc/ H_2O /reflux; l: KOH/ $\text{CH}_3\text{CH}_2\text{OH}$ /reflux, then H_2SO_4 ; m: TFA/ CH_3OH , then $(\text{CH}_3\text{O})_3\text{CH}$

Scheme 3.

a: CAN/AcOH/THF/H₂O; b: 3,4-dimethyl-5-pyrrolin-2-one/KOH/CH₃CH₂OH/N₂, then AcOH; c: KOAc/NaOAc/N₂/135 °C; d: CH₂N₂/CH₃OH

Table 1

Carbon and proton (hydrogen) chemical shifts/ppm and assignments in (CD₃)₂SO for pentapyrrole rubinoid diacids **1–3** and **C-2**^a

Atom No. ^b	1		2		3		C-2 ^b	
	Carbon	Proton	Carbon	Proton	Carbon	Proton	Carbon	Proton
1,24	172.2	–	173.3	–	172.2	–	172.1	–
2,23	123.2	–	123.0	–	123.0	–	122.6	–
2 ¹ ,23 ¹	9.1	1.79	8.9	1.76	9.0	1.75	9.0	1.75
3,22	141.8	–	142.0	–	141.8	–	141.5	–
3 ¹ ,22 ¹	8.8	1.68	8.8	1.75	8.8	1.73	8.2	1.73
4,21	131.2	–	131.9	–	131.9	–	131.9	–
5,20	98.2	5.87	98.3	5.99	98.3	5.87	98.1	5.90
6,19	122.4	–	122.2	–	121.9	–	121.9	–
7,18	123.6	–	124.0	–	123.9	–	122.8	–
7 ¹ ,18 ¹	9.8	1.96	20.0	2.67	23.1	2.38	9.6	2.03
7 ² ,18 ²	–	–	35.9	2.26	24.7	1.33	–	–
7 ³ ,18 ³	–	–	174.4	12.03	28.8	1.27	–	–
7 ⁴ ,18 ⁴	–	–	–	–	31.1	1.49	–	–
7 ⁵ ,18 ⁵	–	–	–	–	34.1	2.15	–	–
7 ⁶ ,18 ⁶	–	–	–	–	174.8	11.89	–	–
8,17	116.0	–	115.0	–	115.0	–	118.8	–
8 ¹ ,17 ¹	10.0	2.45	10.0	1.80	10.0	1.81	19.3	1.96
8 ² ,17 ²	–	2.30	–	–	–	–	34.7	–
8 ³ ,17 ³	–	11.70	–	–	–	–	174.2	–
9,16	129.3	–	129.4	–	129.1	–	128.9	–
10,15	23.0	3.71	23.1	3.71	23.1	3.71	22.5	3.74
11,14	123.9	–	126.7	–	128.4	–	123.5	–
12,13	115.7	–	112.6	–	112.6	–	112.2	–
12 ¹ ,13 ¹	20.0	2.00	10.0	2.02	8.9	2.01	9.1	2.54
12 ² ,13 ²	36.3	–	–	–	–	–	–	2.19
12 ³ ,13 ³	174.6	–	–	–	–	–	–	11.96

Atom No. ^a	1		2		3		C-2 ^b	
Carbon	Carbon	Proton	Carbon	Proton	Carbon	Proton	Carbon	Proton
25,29 NH	-	9.66	-	9.66	-	9.67	-	9.58
26,28 NH	-	10.22	-	10.21	-	10.19	-	10.18
27 NH	-	9.15	-	9.30	-	9.25	-	9.30

^a See Experimental section for integration and multiplicities;

^b see Scheme 4 for numbering system used;

^c data for C-2 taken from Ref. [30]

Table 2

Comparison of ^1H NMR chemical shifts/ppm and assignments of pentapyrrole rubinoid diacids **1** and **3** with 3,18-desvinyl-3,18-dimethylbilirubin-IX α (**D-3**)

Position	Proton	1	3	D-3
5,20	=CH-	6.08	5.87	6.04
α	CH ₂	2.80 – 2.90	1.54 – 1.63 ^a	2.50 – 3.00
β	CH ₂	2.80 – 2.90	2.44 (4H, p) ^{b,c}	2.50 – 3.00
10,15	CH ₂	3.77	3.89, 3.79 ^d	4.07
25,29	lactam N-H	10.68	11.19	10.61
26,28	pyrrole N-H	8.80	10.54	9.15
27	pyrrole N-H	6.91	9.08	–
Acid	CO ₂ H	13.52	–	13.59

^a δ -methylene;

^b ϵ -methylene;

^c $J = 6.8$ Hz;

^d $J = 15$ Hz

Table 3

Comparison of N-H chemical shifts of pentapyrrole rubinoid dimethyl esters **1e–3e** and **C-2e** with **D-3e** (of acids **1–3**, **C-2**, and **D-3**, respectively, of Fig. 1) in CDCl₃ and in (CD₃)₂SO^a

Diester	Chemical shift/ppm in CDCl ₃		Chemical shift/ppm in (CD ₃) ₂ SO	
	Lactam N-H	Pyrrole N-H	Lactam N-H	Pyrrole N-H
1e	11.02	10.39	9.60	10.19
2e	11.18	10.47	9.64	10.21
3e	11.17	10.48	9.66	10.19
C-2e	10.96	10.40	9.64	10.13
D-3e^a	10.44	10.28	9.78	10.41

^aData taken from Ref. [30].

Table 4

Comparison of the propionic ester $-\text{CH}_2-\text{CH}_2-$ segment and C(10) $-\text{CH}_2-$ ^1H NMR splittings in pentapyrrole rubinoid dimethyl esters **1e–3e** and **C-2e** with those of **D-3e** in CDCl_3^a

CH_2	1e	2e	3e	C-2e	D-3e
β	2.92 (2H, ddd)	2.52 (2H, dt)	2.48 (2H, dt)	2.73 (2H, ddd)	2.52 (4H, t)
	$J_{\text{AB}} = 14.3 \text{ Hz}$	$J = 15 \text{ Hz}$	$J = 14 \text{ Hz}$	$J_{\text{AB}} = 11.1 \text{ Hz}$	$J = 7.5 \text{ Hz}$
	$J_{\text{AC}} = 9.3 \text{ Hz}$	$J = 7.5 \text{ Hz}$	$J = 8 \text{ Hz}$	$J_{\text{AC}} = 8.5 \text{ Hz}$	
	$J_{\text{AD}} = 5.5 \text{ Hz}$			$J_{\text{AD}} = 6.1 \text{ Hz}$	
	2.79 (2H, ddd)	2.47 (2H, dt)	2.43 (2H, dt)	2.65 (2H, ddd)	
α	$J_{\text{AB}} = 14.3 \text{ Hz}$	$J = 15 \text{ Hz}$	$J = 14 \text{ Hz}$	$J_{\text{AB}} = 11.1 \text{ Hz}$	
	$J_{\text{BC}} = 6.5 \text{ Hz}$	$J = 7.5 \text{ Hz}$	$J = 18 \text{ Hz}$	$J_{\text{BC}} = 5.6 \text{ Hz}$	
	$J_{\text{BD}} = 10.5 \text{ Hz}$			$J_{\text{BD}} = 9.2 \text{ Hz}$	
	2.51 (2H, ddd)	2.17 (2H, dt)	1.39 (4H, p)	2.22 (2H, ddd)	2.25 (4H, t)
	$J_{\text{AC}} = 9.3 \text{ Hz}$	$J = 17 \text{ Hz}$	$J = 8 \text{ Hz}$	$J_{\text{AC}} = 8.5 \text{ Hz}$	$J = 7.5 \text{ Hz}$
10	$J_{\text{BC}} = 6.5 \text{ Hz}$	$J = 7.5 \text{ Hz}$		$J_{\text{BC}} = 5.6$	
	$J_{\text{CD}} = 15.0 \text{ Hz}$			$J_{\text{CD}} = 13.8 \text{ Hz}$	
	2.46 (2H, ddd)	2.11 (2H, dt)		2.10 (2H ddd)	
	$J_{\text{AD}} = 5.5 \text{ Hz}$	$J = 17 \text{ Hz}$		$J_{\text{AD}} = 6.1 \text{ Hz}$	
	$J_{\text{BD}} = 10.5 \text{ Hz}$	$J = 7.5 \text{ Hz}$		$J_{\text{BD}} = 9.2 \text{ Hz}$	
	$J_{\text{CD}} = 15.0 \text{ Hz}$			$J_{\text{CD}} = 13.8 \text{ Hz}$	
	3.89 (2H, d)	3.87 (2H, d)	3.88 (2H, d)	3.89 (2H, d)	3.83 (2H, s)
	$J = 15.5 \text{ Hz}$	$J = 15.5 \text{ Hz}$	$J = 15.5 \text{ Hz}$	$J = 16 \text{ Hz}$	
	3.81 (2H, d)	3.78 (2H, d)	3.80 (2H, d)	3.70 (2H, d)	
	$J = 15.5 \text{ Hz}$	$J = 15.5 \text{ Hz}$	$J = 15.5 \text{ Hz}$	$J = 16 \text{ Hz}$	

^aChemical shifts in δ /ppm downfield from $(\text{CH}_3)_4\text{Si}$.

^bfor pentapyrrole rubin dimethyl ester **3e**, the 7^1 , 18^1 and 7^2 , 18^2 $-\text{CH}_2-$ groups are ϵ and δ , respectively (not α and β). The relative positions of the methylenes, however, remain consistent for comparison purposes.

Table 5
Comparison of UV-Vis long wavelength absorption^a of pentapyrrole rubinoid diacids **1-3** and **C-2**

Solvent	1		2		3		C-2^b	
	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}
Benzene	420	63000	392	62100	395	62500	390	57700
CHCl ₃	423	44100	394	55250	391	65000	404	42800
CH ₃ CN	413	18100	384	62100	388	60100	387	54600
CH ₃ OH	411	62000	391	59250	391	54300	383	58100
DMF	412	55500	399	61000	411	53400	412	62500
DMSO	411	52070	406	60900	413	52700	409	62450

^a λ_{max} /nm; ϵ_{max} /M⁻¹ cm⁻¹;

^b data from Ref. [30].

Table 6

Comparison of UV-Vis long wavelength absorption^a of pentapyrrole rubinoid dimethyl esters **1e–3e** and **C-2e**

Solvent	1e		2e		3e		C-2e	
	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}
Benzene	393	80300	391	78700	395	80400	388	7200
	445 (sh)	22000	445 (sh)	20500	445 (sh)	21300		
CHCl ₃	390	83000	390	79100	392	83200	385	81900
	440 (sh)	22000	445 (sh)	20500	445 (sh)	21300		
CH ₃ CN	385	81100	384	80400	387	81200	381	83200
	440 (sh)	16700	435 (sh)	17300	440 (sh)	18600		
CH ₃ OH	391	61400	388	67500	390	67500	383	68600
	420 (sh)	48500	420 (sh)	47600	420 (sh)	42300		
DMF	406	63750	402	65800	405	67700	401	63900
DMSO	414	65500	408	67700	412	68900	409	64500

^a λ_{max} /nm; ϵ_{max} /M⁻¹ cm⁻¹;^b from the Ph.D. thesis of Dr. Daniel F. Nogales [50]

Table 7

Comparison of UV-Vis long wavelength absorption^a of verdinoid pentapyrindiones **4–6** and the verdin of **C-2e**

Solvent	4		5		6		C-2e ^b	
	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}	λ_{max}	ϵ_{max}
CHCl ₃	320	54700	320	55600	320	57800	328	57000
	444	21900	456	19300	454	19500	459	21340
	712	7770	736	8750	733	8900	729	8900
CH ₃ OH	318	58100	318	60000	318	61000	321	59500
	446	21200	451	18800	450	18400	456	21000
	704	7760	730	8710	728	8600	726	9800
DMSO	321	56200	321	58000	320	61500	327	58900
	442	25700	448	21700	446	22400	454	24230
	710	7510	728	8200	727	8600	722	9620

^a λ_{max} /nm; ϵ_{max} /M⁻¹ cm⁻¹;

^b from the Ph.D. thesis of Dr. Daniel F. Nogales [50]

Table 8

Circular dichroism and UV-Vis spectra^a for pentapyrrole rubin diacids **1–3** and **C-2** compared to mesobilirubin (MBR) bound to HSA and BSA in 0.1 M tris(hydroxymethyl)aminomethane buffer at pH 7.4^a

Pigment	CD of diacids on HSA		CD of diacids on BSA	
	ϵ_{max} at (λ)	ϵ_{max} at (λ_{max})	ϵ_{max} at (λ)	ϵ_{max} at (λ_{max})
1	–5 (454) 0 (431) +32 (395)	50900 (398)	–10 (457) 0 (442) +43 (404)	50150 (421)
2	+30 (439) 0 (376) –7 (365)	40400 (413)	–15 (404) 0 (385) +6 (372)	42100 (393)
3	–6 (408) 0 (390) +6 (380)	49350 (386)	–5 (415) 0 (392) +6 (389)	48900 (388)
C-2	–5 (441) 0 (427) +10 (399)	42300 (413)	–4 (434) 0 (391) <1 (380)	30000 (400)
MBR ^b	+45 (436) 0 (410) –42 (390)	51200 (434)	–90 (435) 0 (405) +40 (390)	48500 (430)

^a For $2-3 \times 10^{-5}$ M pigment solutions at 20 °C; λ_{max} /nm, ϵ_{max} and $\epsilon_{\text{max}}/\text{M}^{-1} \text{ cm}^{-1}$;

^b data taken from Ref. [50]