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ORIGINAL PAPER

Imploded bilirubins: synthesis and properties of 10-*nor*-mesobilirubin-XIIIα and analogs

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Abstract Six 10-*nor*-bilirubin analogs have been synthesized and investigated. Lacking the C(10) CH_2 group, these linear tetrapyrroles have a bipyrrole core rather than a dipyrrylmethane core and thus a different shape. Whereas the propionic acid groups of bilirubin are well engaged in intramolecular hydrogen bonding to the dipyrrinones, molecular modeling studies of the 10-*nor*-rubins predict that propionic acid chains are too short to engage the CO_2H hydrogen fully in intramolecular hydrogen bonding with the dipyrrinones. Butyric acid chains, however, can and do lead to a stabilized conformation with a bipyrrole dihedral angle of approximately 115°. Spectroscopic studies verify the predictions and vapor pressure osmometry indicates that the 10-*nor*-rubins with butyric acids are monomeric in $CHCl_3$.

Keywords Bipyrrole · Dipyrrinone · NMR

Introduction

Bilirubin (Fig. 1a), the yellow-orange pigment of jaundice and mammalian bile [1, 2] is a linear tetrapyrrole [1] formed in metabolism by opening the heme macrocycle. Along with its analogs that have propionic acids located at C(8) and C(12), bilirubin preferentially adopts a conformation that resembles a half-opened book or ridge-tile that is greatly stabilized by a network of intramolecular hydrogen bonds linking propionic acid groups to the dipyrrinone units (Fig. 1b). The location of the propionic acids is important, and the nature and position of the remaining *β*-substituents less so, e.g., analogs such as the synthetic pigment, mesobilirubin-XIIIα (Fig. 1c) are intramolecularly hydrogen-bonded. Such conformation-determining hydrogen bonding, which depends on the unusually strong attraction of dipyrrinone and carboxylic acid [3–6], dominates the pigment's shape, properties and metabolism [7–9]. The importance of the C(10) CH_2 group [10], and even a C(10)-S or Se [11–13], to the pigment's shape cannot be overstated: with two bond rotational degrees of freedom between C(10) and the conjoined approximately planar dipyrrinones, a large number of conformations is implicitly possible; yet, in order to minimize nonbonded steric interactions, few are low-energy, and those are stabilized as a ridge-tile shape, with C(10) at the crease, by intramolecular hydrogen bonding [10]. With more bond rotational degrees of freedom—as in a homorubin, where C(10)–CH_2 is replaced by a –CH_2–CH_2– unit—the molecule folds into a cup shape [14]. Also, in exploded bilirubins, where the C(10) CH_2 is replaced by –C≡C– or –C≡C–C≡C–, the pigments are linear in shape, even with intramolecular hydrogen bonding [15]. Such shape-altered bilirubin analogs are important to our understanding of the molecular mechanisms at the various steps of hepatic elimination [7–9, 11–15].

In the current study, we have prepared bilirubin analogs lacking a C(10) CH_2: imploded rubins with a bipyrrole core replacing the standard dipyrrylmethane. In the following, we discuss the syntheses of **1**–**6**, their stereochemistry and properties (Structure 1).

Results and discussion

Synthesis aspects

Retrosynthetic analysis indicated two feasible routes (Fig. 2a) toward the synthesis of 10-*nor*-rubins with a

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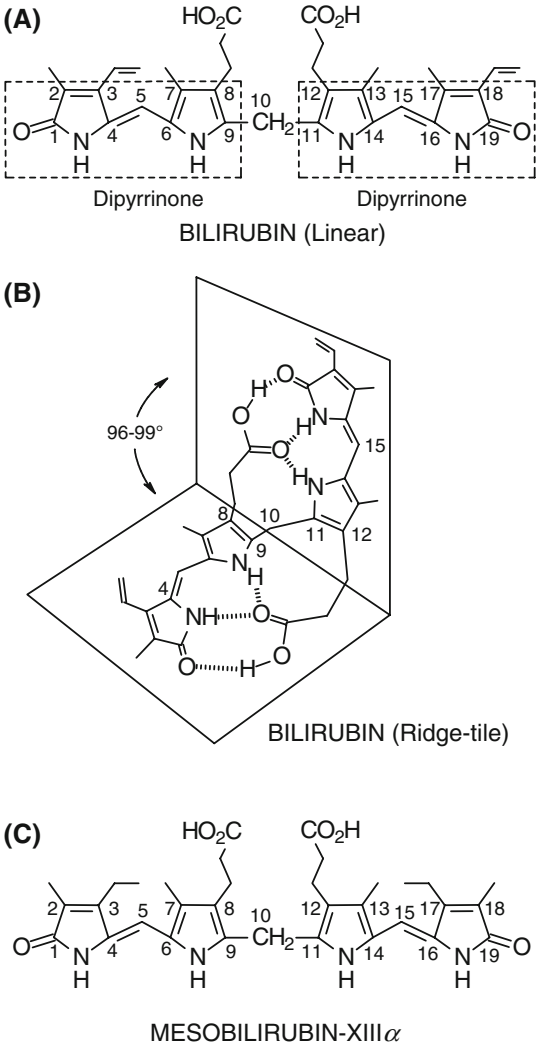
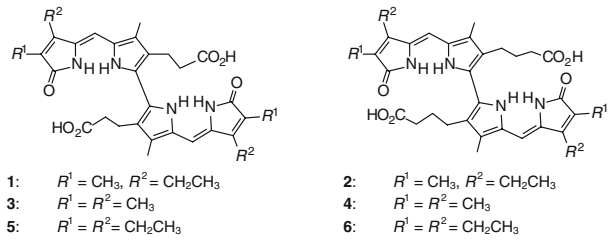


Fig. 1 **a** Bilirubin as a linear tetrapyrrole. **b** Bilirubin in its most stable, intramolecularly hydrogen-bonded conformation. Only one of two enantiomers is shown. **c** A synthetic bilirubin analog, mesobilirubin-XIIIα in a linear projection. The ridge-tile conformation is also the most stable



Structure 1

2,2′-bipyrrole core: (1) a "2 + 2" approach [1, 16] involving bipyrrole-formation coupling of 9-H dipyrrinones, and (2) a "1 + 2+1" approach [16] wherein the 2,2′-bipyrrole nucleus was extended at each end through standard dipyrrinone-forming coupling reactions. The first has already been reported in the literature [17, 18], and (given the availability of 9-H dipyrrinones in our lab) suggested an attractive, straightforward route. However, both the 9-H dipyrrinone acid and ester (Fig. 2b) failed to couple under a variety of established coupling conditions [1, 17], and the starting material was recovered. Similarly, the iodo-ester of Fig. 2b failed to couple under standard Cu-catalyzed bipyrrole coupling methods [1, 19–21], and the 9-H dipyrrinone was recovered. Apparently, the presence of the propionic acid or ester group at C(8) is involved in quenching the radical intermediate(s), because coupling works well with a C(8)–CH_3 group.

Given these unexpected difficulties, we focused on route 2 of Fig. 2a, the "1 + 2+1" synthesis that expands from the bipyrrole nucleus [22] and which required the preparation of the new bipyrroles **7**, **9**, **17** and **18** of Fig. 2a. These bipyrroles thus became the core component targets in the syntheses of **1**–**6**, the final steps of which are outlined in Scheme 1 showing: (a) reaction of tetraacid **7** or **17** with 5-bromomethylene-4-ethyl-3-methyl-3-pyrrolin-2-one [23] to give dimethyl esters, e.g., **1e** and **2e**, which can be saponified to **1** or **2**—a standard dipyrrinone-forming procedure [16]; and (b) condensation of bipyrrole–dialdehyde **9** or **18** with 3,4-dimethyl-3-pyrrolin-2-one to give **3** or **4**, or with 3,4-diethyl-3-pyrrolin-2-one [24–27] to give **5** or **6**—a well-developed dipyrrinone- and bilirubin-forming reaction [1, 25, 28–30]. The condensations shown in (a) above give dimethyl esters, which are saponified to diacids; those of (b) gave diacids directly.

We first set out to prepare *nor*-mesobilirubin-XIIIα (**1**), which required bipyrrole tetra acid intermediate **7**, the synthesis of which was designed from multifunctional

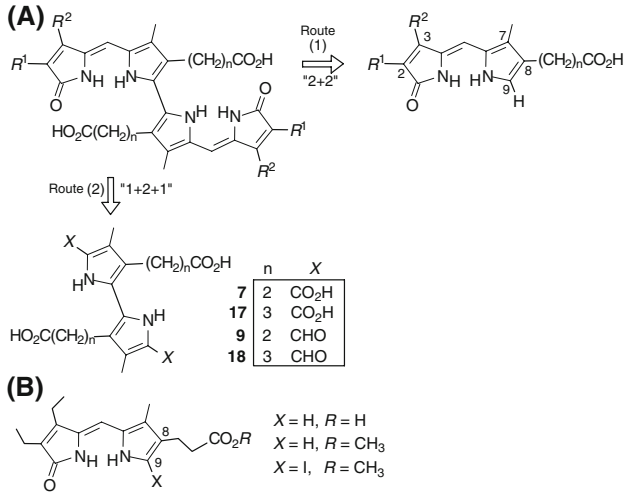
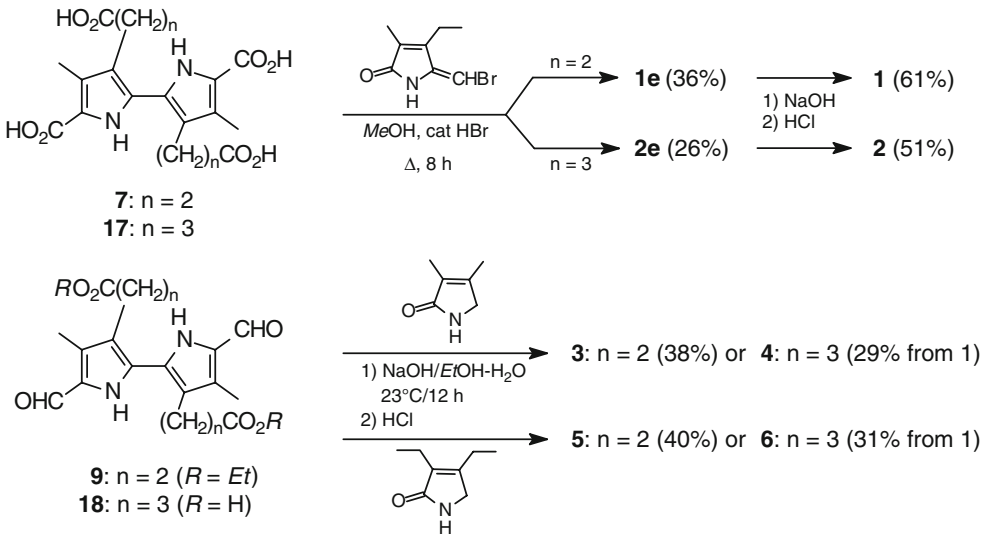


Fig. 2 **a** Possible retrosynthetic routes to 10-*nor*-bilirubin analogs: *(1)* by coupling two 9-H dipyrrinones (R^1 and R^2 = CH_3 or CH_2CH_3) and *(2)* by dipyrrinone-forming reactions on a bipyrrole core (X = H or CHO). **b** Dipyrrinones used in failed coupling attempts in route *(1)* of **a**

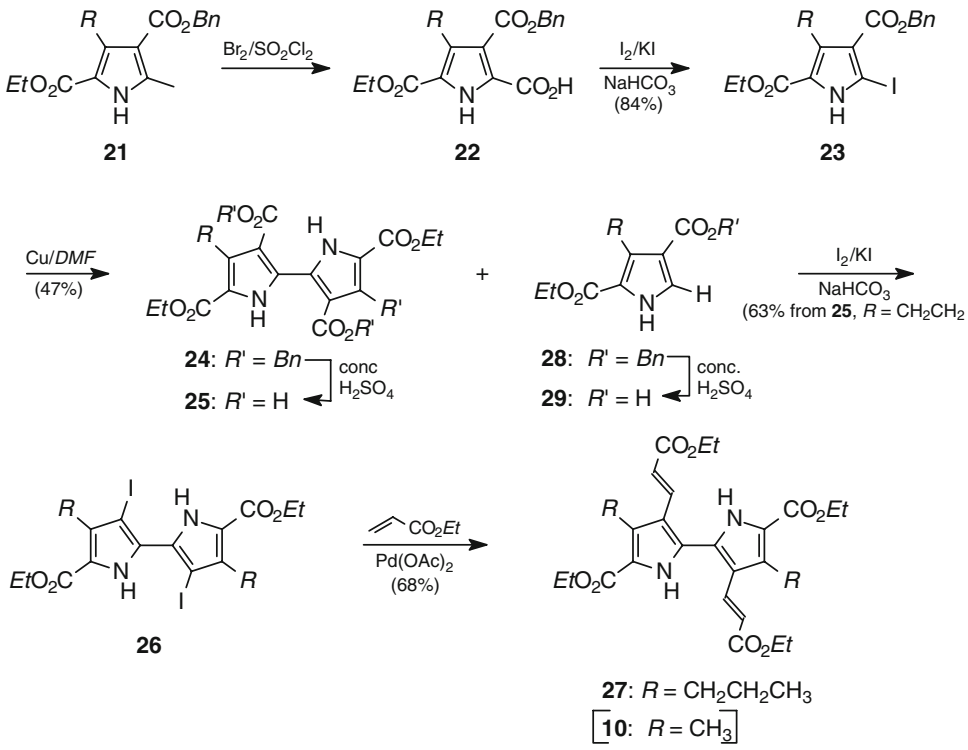
Scheme 1



monopyrrole **22** ($R = CH_3$). The latter had been prepared from diester **21** ($R = CH_3$) (Scheme 2) and converted to an α-iodopyrrole (**23**, $R = CH_3$), which was viewed as suitable for Cu-catalyzed coupling to bipyrrole **24** ($R = CH_3$) [31]. Selective de-esterification (debenzylation) of **24** to **25** ($R = CH_3$) was planned, followed again by decarboxylative iodination to give **26**, the iodines of which would be replaced by acrylate esters via a Heck coupling reaction [32]. However, this plan presented difficulties because the diacid intermediate **25**, when $R = CH_3$, was highly insoluble [31] in characterization and Heck reaction solvents. In order to overcome these solubility problems, we successfully carried out the entire reaction sequence of Scheme 2 with $R = CH_2CH_2CH_3$, which improved the solubility of the pyrrolic compounds sufficiently in standard organic solvents [31, 33] so that debenzylation of **24**–**25** was smoothly achieved.

Bipyrrole-forming reactions involving Cu-catalyzed coupling of iodopyrroles can be capricious and depend, inter alia, on the nature of the Cu and the activity at its surface [1, 17, 18, 20, 21]. The deiodinated monopyrrole, e.g., **28** ($R = CH_2CH_2CH_3$), is a typical by-product, and at

Scheme 2



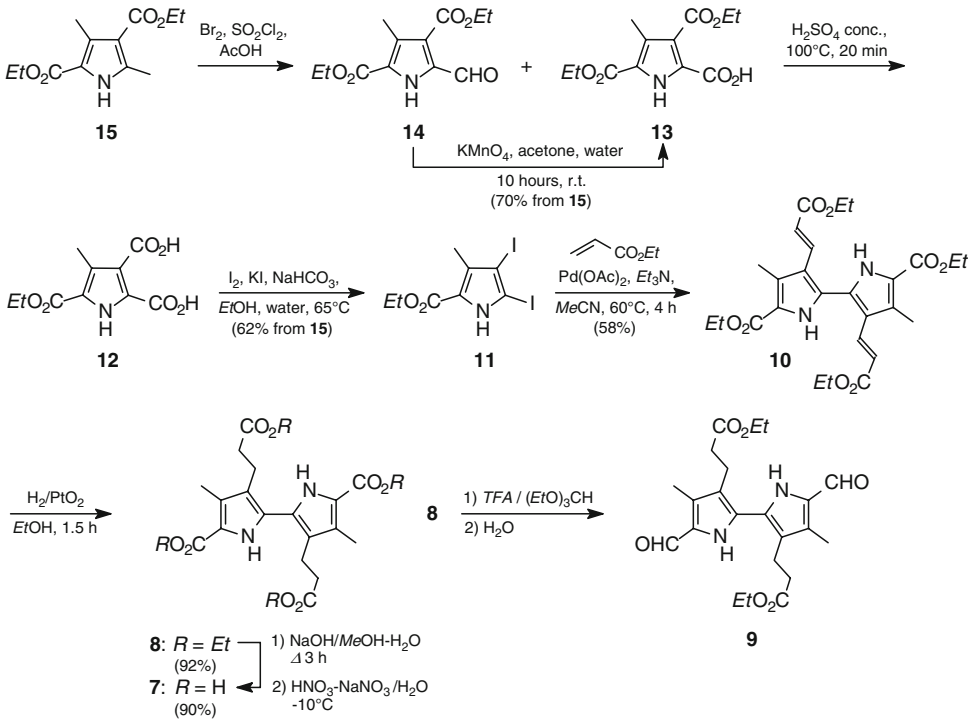
times even the major product. It was through this by-product that we serendipitously discovered a shortcut to **27** involving a new bipyrrole coupling reaction [33] that considerably shortened the sequence and facilitated the project by providing a route to **10** that avoided poorly soluble **25** ($R = CH_3$) (vide infra).

As outlined in Scheme 2 ($R = CH_2CH_2CH_3$), the conversion of **21**–**27** became routine, with acceptable yields at each step, except for the Cu-catalyzed bipyrrole coupling reaction of **23**–**24**. Here the yield of **24** was variable, with the deiodinated monopyrrole **28** being a major by-product. It was normally separated from **24** before the latter was carried forward in 2–3 steps (debenzylation, decarboxylation, iodination) to form diiodide **26**. Diiodo-bipyrrole **26** was then converted via a Heck coupling [32] with ethyl acrylate smoothly to afford **27**, which, after reduction of the acrylate ester carbon–carbon double bonds, was to become the core component of the "1 + 2 + 1" synthesis of 10-*nor*-rubins. However, they would be rubins with *n*-propyl groups in place of the typical methyl groups on the middle pyrrole rings.

Serendipitously, in one run, not **24** but the separated, deiodinated monopyrrole by-product **28** ($R = CH_2CH_2CH_3$) was accidentally carried forward with debenzylation to **29**, iodination, the $Pd(OAc)_2$-catalyzed reaction with ethyl acrylate, and this led to the fortuitous discovery of a new bipyrrole coupling reaction [33]. At that time the conversion of **24**–**27** ($R = CH_2CH_2CH_3$) had become so routine that we were isolating but no longer characterizing the two bipyrrole intermediates (**25** and **26**) in the two-step synthetic path from **25** to **27**. Thus, by mistake, in one run the monopyrrole by-product **28** ($R = CH_2CH_2CH_3$) was run through the same two steps, and we obtained **27** in good yield. At first we failed to realize that anything unusual had happened, until we discovered that **28** (and not the intended **24**) had been mistakenly carried forward—and actually gave **27**. Apparently **28** had been converted to **29** and on to its 4,5-diiodo monopyrrole analog, and it was in fact this monopyrrole that had undergone a one-pot bipyrrole coupling together with a Heck reaction [32], with both coupling and Heck reactions using the same $Pd(OAc)_2$ catalyst. Subsequent test reactions showed that treatment of the 5-iodo derivative of **28** under the same reaction conditions (either with or without ethyl acrylate) failed to give a bipyrrole, which was not surprising, as the $Pd(OAc)_2$ catalyst is not known to convert iodopyrroles to bipyrroles. We thus suspect that first a Heck reaction occurred at C(4) of the diiodo-pyrrole to introduce the ethyl acrylate group, which when coordinated to the Pd directed a bipyrrole coupling reaction. A similar reaction with 1,2-diiodobenzene failed to give a biphenyl.

The conversion of a 4,5-diiodopyrrole in one step to a 4-substituted-α,α′-bipyrrole thus represents a new and efficient bipyrrole coupling reaction. This discovery bypasses the stepwise procedure of Scheme 2 and led to our abandoning it, along with all of the *n*-propyl pyrrole chemistry, because the diiodo-pyrrole coupling substitution circumvents the insolubility problem posed by intermediate **25** ($R = CH_3$) and thus opened up a facile route (Scheme 3) to core bipyrroles **8** and **9** used in the syntheses of

Scheme 3



10-*nor*-rubins **1**–**3**. In the most direct path to the desired core bipyrroles **7** and **9** (outlined in Scheme 3), multifunctional **22** (R = CH_3) of Scheme 2 was not required; rather, the synthesis starts from the readily available diethyl 3,5-dimethyl-1*H*-pyrrole-2,4-dicarboxylate **15** [23]. Conversion of its α-CH_3 to α-CO_2H using Br_2/SO_2Cl_2 was often incomplete and gave a mixture of α-CHO **14** and α-CO_2H **13**. However, the former could be converted smoothly to the latter by reaction with dilute $KMnO_4$, and acid **13** was readily and selectively de-esterified to diacid **12** using conc. H_2SO_4. The latter diacid afforded 4,5-diiodopyrrole **11** following reaction with I_2/KI in aqueous $NaHCO_3$ [15], and **11** coupled to give bipyrrole **10** in good yield in a one-pot reaction with ethyl acrylate in the presence of the Pd(O*Ac*)$_2$ catalyst. Catalytic hydrogenation of **10** reduced the acrylic ester groups to propionic (**8**), and saponification of **8** gave **7**, which could be converted readily to dialdehyde **9**. From **7**, we prepared **1**; from **9** we prepared **3** and **5** (see Scheme 1).

Synthesis of **2**, **4**, and **6** was also desired because molecular modeling studies indicated that a butyric acid chain would be a better fit in intramolecular hydrogen bonding to the opposing dipyrrinones. Preliminary studies showed that we could introduce a terminal acetylene by a Sonogashira reaction [34] of diiodo-bipyrrole **26** (R = $CH_2CH_2CH_3$) [31]. The discovery that 4,5-diiodo-monopyrrole **11** would yield the bis-acrylate bipyrroles **27** (R = $CH_2CH_2CH_3$) and **10** (R = CH_3) (Scheme 3) prompted us to attempt, successfully, a Sonogashira reaction [33] of **11** with methyl 3-butynoate (Scheme 4). Indeed the combined substitution-bipyrrole coupling reaction worked nicely under Sonogashira conditions, giving diacetylene bipyrrole **20** in good yield. The latter was reduced to give bis-butyric ester bipyrrole **19**, which was converted to **17**, and then to **18**, as shown in Scheme 4. From **17**, we were able to prepare **2**, and from **18** we prepared **4** and **6**, as outlined in Scheme 1.

Structures and ^{13}C NMR spectroscopy

The constitutional structures of **1**–**6** follow from their syntheses (Schemes 1, 2, 3, 4) and are confirmed by their ^{13}C NMR spectra. Most notably, in comparing **1** to mesobilirubin-XIIIα (Fig. 1c), the unique resonance at 23.3 ppm corresponding to the C(10) CH_2 group is absent, as it is in **2**–**6** (Table 1). Similarly, in the ^1H NMR spectra, the unique resonance near 4 ppm corresponding to the C(10) CH_2 is also absent in **1**–**6**. One may find all of the corresponding dipyrrinone ring carbon resonances of mesobilirubin-XIIIα in the bis-dipyrrinones **1**–**6**, and the ^{13}C resonances of the β-substituents of **1** match up well with those of mesobilirubin-XIIIα [29] and our recent assignments for xanthobilirubic acid [35]. Similar correlations for **3** and **5** may be found. The ^{13}C NMR signals from 10-*nor*-butyric acid rubins **2**, **4** and **6** also correlate well to mesobilirubin-XIIIα and its bis-butyric acid analog. Differences between certain chemical shifts from **1** to **6** versus mesobilirubin-XIIIα are found mainly in the pyrrole ring resonances: C(9)/C(11) are 3–4 ppm more deshielded in **1**–**6**, C(8)/C(12) are ~7 ppm more deshielded, and C(7)/C(13) and C(6)/C(14) are ~8 ppm more deshielded. Smaller deshieldings (1–2 ppm) are found in the pyrrolinone rings of **1**–**6**. All are apparently due to absence of the C(10) CH_2.

Scheme 4

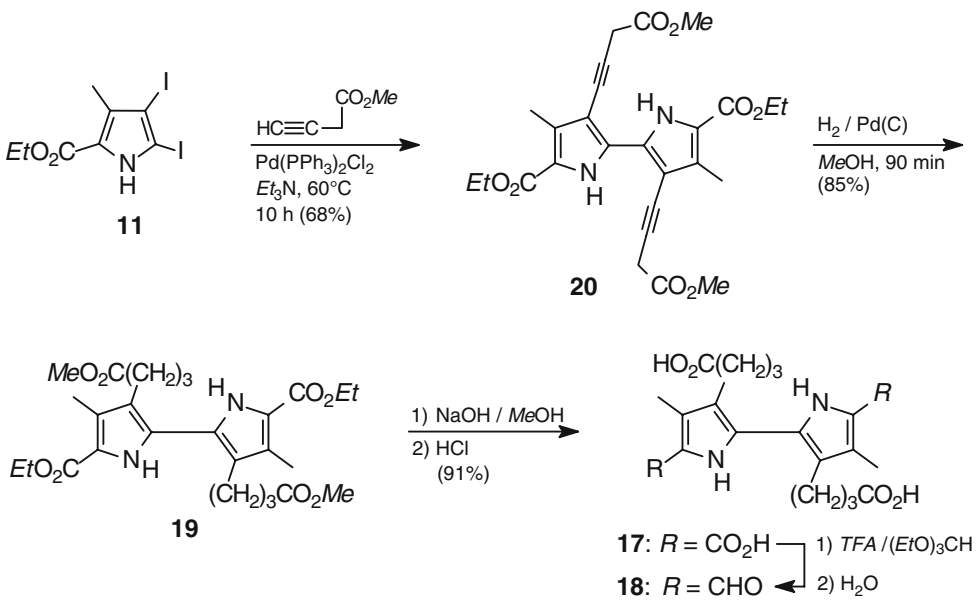


Table 1 ^{13}C NMR chemical shifts of 10-*nor*-mesobilirubins **1**–**6** and mesobilirubin-XIIIα (MBR) in $(CD_3)_2SO$ solvent

Carbon[a]		**1**	**3**	**5**	**2**	**4**	**6**	MBR[a]
1/9	C=O	172.9	172.7	172.8	172.1	172.2	172.1	171.9
2/18	–C=	123.5	124.3	124.4	124.2	123.3	124.9	122.9
3/17	–C=	148.5	148.5	148.6	146.2	146.2	146.1	147.1
4/16	–C=	129.2	129.2	129.2	130.9	131.1	130.9	127.8
5/15	–CH=	98.4	98.4	98.4	99.1	99.2	99.0	97.7
6/14	–C=	129.99	129.89	130.01	127.1	127.2	127.1	122.4
7/13	–C=	129.95	129.98	129.96	126.2	128.7	126.3	122.0
8/12	–C=	126.3	126.2	126.3	125.1	125.1	125.0	119.3
9/11	–C=	133.3	134.0	134.1	137.5	137.5	137.4	130.4
$2^1/18^1$	CH_2/CH_3	8.0	8.4	19.2	8.2	8.2	16.1	8.1
$2^2/18^2$	CH_3	–	–	13.7	–	–	14.4	–
$3^1/17^1$	$CHCH_3$	19.6	19.6	19.7	17.2	9.4	18.3	17.2
$3^2/17^2$	CH_3	15.0	–	14.0	14.9	–	15.8	14.8
$7^1/13^1$	CH_3	9.6	9.6	9.7	9.3	9.5	9.3	9.1
$8^1/12^1$	CH_2	21.2	21.2	21.2	20.8	21.0	20.9	19.2
$8^2/12^2$	CH_2	33.7	33.8	33.8	23.9	24.0	24.0	34.6
$8^3/12^3$	CH_2	–	–	–	34.6	34.7	34.6	–
$8^3/12^3$	CO_2H	174.2	174.0	174.0	174.2	174.3	174.2	174.1
10	CH_2	–	–	–	–	–	–	23.3

In δ, ppm downfield from $(CH_3)_4Si$

[a] Chemical shifts from [29] with assignments for $2^1/18^1$ CH_3 and $7^1/13^1$ CH_3 assigned according to our most recent studies [35]

Solution and chromatographic properties

The 10-*nor*-rubins **1**, **3** and **5** with propionic acid substituents are more polar and less soluble in $CHCl_3$ than the corresponding 10-*nor*-rubins **2**, **4** and **6** with butyric acid substituents. Increased polarity in rubins typically signifies decreased effectiveness of intramolecular hydrogen bonding, which we attribute, at least in part, to the longer hydrogen bonding distances in **1**, **3** and **5** than in **2**, **4** and **6**. In bilirubin and mesobilirubin-XIIIα, propionic acid chains enjoy optimal intramolecular hydrogen bonding (Fig. 1c) [36–40], but butyric acid chains are also found to possess the right geometry for engaging the dipyrrinones in hydrogen bonding [41–45]. When the bilirubin acid chains are as short as acetic or as long as pentanoic and hexanoic, rather counterintuitively the pigment polarity increases [45]. Generally, when the acid chain lengths are mismatched for effective intramolecular hydrogen bonding, as in bilirubins with pentanoic through octanoic acids at C(8) and C(12), counterintuitively, the pigments typically exhibit increased polarity (on TLC), decreased solubility in $CHCl_3$, and increased solubility in dilute aqueous bicarbonate.

Unlike their bright yellow parent, mesobilirubin-XIIIα, 10-*nor*-rubins **1**–**6** are yellow-orange solids that form yellowish solutions. Analogs **1**, **3** and **5** are insoluble in most nonpolar organic solvents such as $CHCl_3$ and CH_2Cl_2. In contrast, **2**, **4** and **6** exhibit good solubility in $CHCl_3$ and CH_2Cl_2, an indication that the latter are less polar than the former. Consistent with such polarity, on silica gel TLC using 5% by vol CH_3OH in CH_2Cl_2 as eluent, **2**, **4** and **6** have an $R_f \sim 0.75$–0.78, and **1**, **3** and **5** have an $R_f \sim 0.29$–0.30, under the same conditions where mesobilirubin-XIIIα exhibits an $R_f \sim 0.89$. As with bilirubin and mesobilirubin-XIIIα, **2**, **4** and **6** are not extracted into 5% (or saturated) aqueous sodium bicarbonate from chloroform; however **1**, **3** and **5** are extracted. Clearly, replacing the central $–CH_2–$ of mesobilirubin-XIIIα by C–C has a major impact on the chloroform/bicarbonate partition coefficient of **1**, **3** and **5**, but it does not have a large effect when the chain lengths are extended to butyric. Taken collectively, these data suggest the presence of effective intramolecular hydrogen bonding in **2**, **4** and **6**, but not in **1**, **3** and **5**, as predicted by CPK molecular models, and as designed.

Intramolecular hydrogen bonding

In 10-*nor*-bilirubins, removal of the C(10) CH_2 group removes one rotational degree of freedom of the dipyrrinones relative to one another, allowing motion solely about the bipyrrole sp^2–sp^2 C–C bond. Inspection of CPK models indicates that an *anticlinal* (*ac*) conformation about the

C(9)–C(11) bond is the one most likely to lead to intramolecular hydrogen bonding with alkanoic acids situated at C(8) and C(12), as in bilirubin. In such a conformation, the C(8)/C(12) propionic CO_2H of **1**, **3** and **5** can engage in hydrogen bonding with the opposing dipyrrinone's NHs and the lactam only if the dipyrrinones are twisted about the C(5)–C(6) and C(14)–C(15) bonds. Otherwise the O=C distance is too long for hydrogen bond formation. Fully engaged hydrogen bonding between the lactam C=O and the acid OH requires a longer acid chain than propionic. The cost of twisting within the dipyrrinones destabilizes the system by about 17–20 kJ/mol. Ideally, as in bilirubin, the dipyrrinones would be planar. And for planar dipyrrinones, the butyric acids of **2**, **4** and **6** appear to have the ideal minimum chain length.

Consistent with the predictions based on acid chain length, the ^{1}H NMR spectra of **2**, **4** and **6** in $CDCl_3$ reveal NH and COOH chemical shifts (Table 2) very similar to those of mesobilirubin-XIIIα [29] and characteristic of intramolecular hydrogen bonding (Fig. 1b). In clear contrast, the corresponding chemical shifts of **1**, **3** and **5** diverge significantly and suggest a different type of hydrogen bonding. Dipyrrinones are known to participate avidly in hydrogen bonding, especially to carboxylic acids, but also to each other, with association constants of approx. 3×10^4 M^{-1} at 25 °C in $CDCl_3$. At very high dilution, dipyrrinone monomers show pyrrole and lactam NH chemical shifts of ~8 ppm, but more commonly seen dipyrrinone dimers exhibit lactam and pyrrole NH signals at ~11 and ~10 ppm, respectively, in the ^{1}H NMR. In contrast, when the dipyrrinone is engaged in hydrogen bonding to a COOH group, the lactam and pyrrole chemical shifts are typically ~10.5 and ~9 ppm. The more shielded pyrrole NH signal appears to be diagnostic of intramolecularly hydrogen-bonded bilirubin-type molecules. In bilirubin and mesobilirubin-XIIIα, the lactam NH (~10.6 ppm) and pyrrole NH (~9.2) chemical shifts as well as the deshielded COOH (~13.6 ppm) chemical shift are representative examples. Similar chemical shifts have been observed in a diverse array of other intramolecularly hydrogen-bonded rubins [11–13, 24, 28–30, 46–50], even in "exploded" rubins [15, 51], as well as in semirubins [3] and hemirubins [52, 53], where only one dipyrrinone is present. Table 2 shows the excellent coincidence of lactam, pyrrole and carboxylic acid ^{1}H NMR chemical shifts of **2**, **4** and **6** in $CDCl_3$ with those of mesobilirubin-XIIIα. On this basis, one might conclude that these 10-*nor*-rubins are intramolecularly hydrogen-bonded. Table 2 also clearly shows differences between **1**, **3** and **5** relative to **2**, **4** and **6** in $CDCl_3$. The NH chemical shifts of the former set are very much like those seen for intermolecularly hydrogen-bonded dipyrrinone dimers, thus suggesting more strained dipyrrinone to carboxylic acid hydrogen bonding, and possibly dipyrrinone-to-dipyrrinone dimers or oligomers, rather than monomers.

Thus, on the basis of these data, one might assume carboxylic acid to dipyrrinone hydrogen bonding in **2**, **4** and **6**—as predicted by molecular modeling, but not in **1**, **3** and **5**. The hydrogen bonding observed in the butyric acid

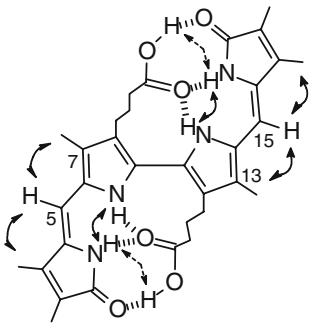


Fig. 3 Strong NOEs found for **4** in $CDCl_3$ are shown by *curved*, *double-headed arrows*. Weaker NOEs are shown by *curved*, *dashed arrows*. Similar NOEs are seen in **2** and **6**

Table 2 Comparison of carboxylic acid OH and lactam and pyrrole NH ^{1}H NMR chemical shifts of 10-*nor*-bilirubins **1**–**6** and mesobilirubin-XIIIα (MBR) in $CDCl_3$ and $(CD_3)_2SO$

Compound	δ (ppm) in $CDCl_3$[a]			δ (ppm) in $(CD_3)_2SO$[a]		
	Lactam NH	Pyrrole NH	COOH	Lactam NH	Pyrrole NH	COOH
1	11.23	10.13	11.87	9.95	10.23	11.91
3	11.15	10.11	11.90	9.90	10.31	11.98
5	11.29	10.39	11.81	9.95	10.25	11.88
2	10.77	9.30	13.35	9.94	10.71	11.98
4	10.75	9.22	13.44	9.75	10.91	11.91
6	10.69	9.44	13.60	9.90	10.39	11.88
MBR[b]	10.58	9.15	13.64	9.77	10.31	11.89

[a] δ, ppm relative to $(CH_3)_4Si$ at 23 °C for 10^{-3} to 10^{-4} M solutions

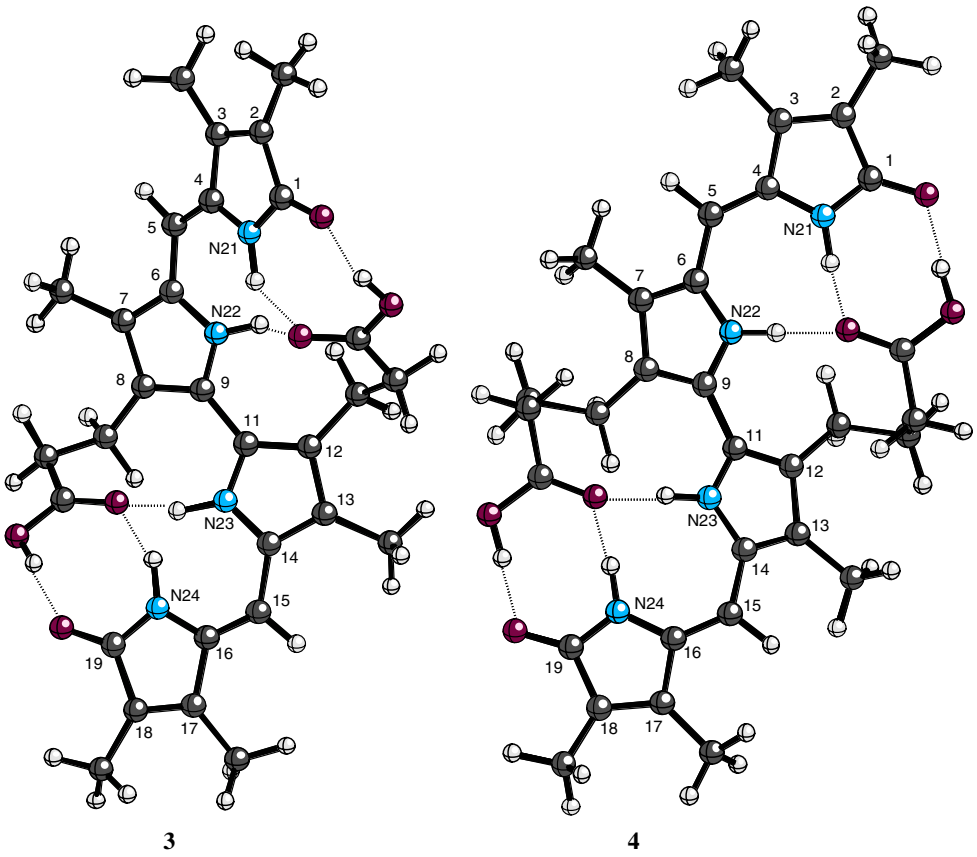
[b] Values from [29]

10-*nor*-rubins is further supported by NOEs between the COOH and lactam NH (Fig. 3) and the additional, stronger NOEs, between the lactam and pyrrole NHs and between the C(5)/C(15) H and the C(7)/C(13) CH_3 support the *syn-Z*-conformation of the pigments. Pigments **1**, **3** and **5** were insufficiently soluble in $CDCl_3$ for NOE studies.

Additional evidence in support of an intramolecularly hydrogen-bonded structure for **2**, **4** and **6** comes from vapor pressure osmometry (VPO) determinations [3, 54] of molecular weight in $CHCl_3$ solution. For **4** (FW = 574.7) and **6** (FW = 630.8), we determined molecular weights of 588 ± 10 and 649 ± 10, respectively. On this basis, we take solutions of **2**, **4**, and **6** in $CHCl_3$ to be monomeric. Unfortunately, pigments **1**, **3** and **5** were insufficiently soluble in $CHCl_3$ for VPO studies.

Conformation analysis from molecular dynamics

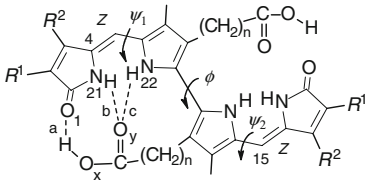
Independent rotations of the approximately planar, thermodynamically most stable *syn-Z*-dipyrrinones of **1**–**6** about the bipyrrole bond (ϕ) lead to an infinite number of conformations, including two high-energy limiting cases [as determined by the Sybyl force field (see the footnote near the start of the "Experimental" section)] where the dipyrrinones lie coplanar: the *syn* and *anti*. Lying between these extremes is a low-energy conformation twisted from coplanarity to minimize nonbonded steric interactions and stabilized by intramolecular hydrogen bonding, given sufficient length of the alkanoic acid substituents at C(8) and C(12). Upon comparing **1**, **3** or **5** and **2**, **4** or **6**, molecular models show a better match for intramolecular hydrogen bonding with butyric acids (**2**, **4**, **6**) than with propionic acids (**1**, **3**, **5**): both CO_2H groups of the first set are fully engaged in intramolecular hydrogen bonding to an opposing dipyrrinone; in **1**, **3** or **5** the CO_2H group is less well engaged. Conformational analysis by molecular dynamics calculations using Sybyl (see the footnote near the start of the "Experimental" section) gave an energy-minimum conformation of **2**, **4** and **6** in which the 114° interplanar torsion angle (ϕ = N–C–C–N) of the *anticlinal* bipyrrole unit is some 35° smaller than the ϕ = 148° angle of **1**, **3** and **5**. In **2**, **4** and **6**, both butyric acid groups are fully engaged in intramolecular hydrogen bonding to an opposing dipyrrinone (Fig. 4), but for **1**, **3** and **5**, while the propionic acid C=O hydrogen bonds equally effectively [see d(b) and d(c) of Table 3], in the latter, the CO_2H hydrogen lies ~0.1 Å further removed from the lactam C=O than in **2**, **4**, **6** and mesobilirubin [see d(a) of Table 3]. Note that the computed hydrogen bond distances (Table 3) of **2** and **4** and **6** are nearly identical to those in mesobilirubin. It is also important to note here that, as with other bipyrroles, **1**–**6** adopt equi-energetic enantiomeric conformations.



3 **4**

Fig. 4 Ball and stick (see the footnote near the start of the "Experimental" section) representations of the minimum energy conformations of **3** (bis-propionic acid), **4** (bis-butyric acid) and 10-*nor*-rubins, as determined by molecular mechanics computations using the Sybyl forcefield. The relevant torsion angles (ϕ and ψ, in degrees) are: ϕ = (22-9-11-12), ψ_1 = (4-5-6-22), and ψ_2 = (23-24-15-16). In **3**: ϕ = 110°, ψ_1 = 20°, and ψ_2 = 9°; in **4**: ϕ = 118°, ψ_1 = 19°, and ψ_2 = 6°. Significantly, the O–H···O (lactam) bond angle in **3** is 35°, whereas in **4** it is 173°—indicating that the OH of **3**, but not **4**, is turned away from proper intramolecular hydrogen bonding, and consistent with the finding that the CO_2H···O (lactam) hydrogen bond is long in **3**; whereas in **4** it is the same as in bilirubin and mesobilirubin (Table 3). The computed results for **1** and **5**, and **2** and **6** are similar to those of **3** and **4**, respectively

Table 3 Comparison of computed torsion angles and nonbonded distances of 10-*nor*-rubins **3–6** and mesobilirubin-XIIIα (MBR) in their most stable conformations



	Torsion angles (°)						Distances (Å)			H-bond angles (°)		
	n	R^1	R^2	ϕ	ψ_1	ψ_2	d(a)	d(b)	d(c)	OH···O	N_{22}–H···O	N_{21}–H···O
3	2	Me	Me	148	25	25	1.63	1.53	1.55	161	145	143
5	2	Et	Et	148	25	25	1.63	1.53	1.55	161	145	144
4	3	Me	Me	114	24	26	1.52	1.56	1.61	171	142	146
6	3	Et	Et	114	24	25	1.51	1.57	1.60	169	142	148
MBR	2	Me	Et	64	17	17	1.53	1.59	1.56	170	163	154

From Sybyl vers. 7.0 run on an SGI workstation. The 0° torsion angles are those where the relevant nitrogens are *syn*-periplanar

Table 4 Solvent-dependence of the UV–visible spectral data of 10-*nor*-rubins **1–6** with mesobilirubin-XIIIα (MBR)

Compound	ε^{max} (dm^3 mol^{-1} cm^{-1}) [λ^{max} (nm)]					
	Benzene	$CHCl_3$	$(CH_3)_2CO$	CH_3OH	CH_3CN	$(CH_3)_2SO$
1	39,200 (450)	38,400 (455)	37,900 (454)	38,200 (450)	37,600 (449)	36,400 (450)
3	38,200 (452)	40,100 (454)	39,800 (456)	37,100 (451)	37,000 (449)	36,000 (454)
5	37,000 (456)	39,800 (457)	38,000 (455)	36,600 (452)	39,900 (452)	34,800 (453)
2	38,900 (453)	38,700 (455)	37,500 (455)	37,200 (452)	40,100 (452)	37,700 (456)
4	35,950 (456)	39,450 (456)	36,950 (451)	39,300 (453)	39,150 (451)	30,250 (453)
6	40,000 (455)	37,800 (454)	38,500 (452)	38,900 (454)	36,500 (452)	37,250 (452)
MBR	49,900 (439)	50,300 (431)	49,700 (427)	50,600 (425)	49,000 (425)	52,500 (428)

Shoulders (or) inflections were determined by first- and second-derivative spectra at 22 °C in concentrations of $\sim 1.4 \times 10^{-5}$ M

From the computed relative energies of the most stable conformations and the *syn* and *anti*-periplanar bipyrrole conformations ($\phi = 0°$ and $\phi = 180°$, respectively), one can compute the lowest-energy barriers to racemization via the *ap* bipyrrole conformers as ~105 kJ/mol for **1**, **3** and **5** and ~120 kJ/mol for **2**, **4** and **6**, suggesting that the global minimum conformations of the former are less stable than those of the latter by ~15 kJ/mol.

Conformation from UV–visible spectra

Additional evidence regarding the conformation of **1–6** comes from solvent-dependent UV–visible spectra. Compared with their "parent" rubin (mesobilirubin-XIIIα, with broad absorption near 430 nm and a shoulder near 395 nm in most solvents), the 10-*nor*-rubins showed (Table 4) a broad band centered near 450 nm, thus accounting for their more orange color. Over a wide range of solvents of varying polarity and hydrogen bonding ability (benzene, chloroform, acetone, methanol, acetonitrile, and dimethylsulfoxide), the UV–visible long wavelength absorbance, i.e., λ_{max}, of **1–6** show much less solvent-dependence than of mesobilirubin-XIIIα (Table 4), indicating very little conformational change over the range of solvent polarity. The absorptivity of the long wavelength band is smaller than in mesobilirubin-XIIIα.

In the UV–visible spectrum of mesobilirubin-XIIIα, the long wavelength absorption near 430 nm originates from exciton coupling [10, 55], from the electric dipole–electric dipole transition moment interaction of the two dipyrrinone chromophores. The dipyrrinone chromophores of this study typically exhibit UV–visible λ_{max} ~ 400–420 nm for their long-wavelength absorption band, with the electric transition dipole moment lying along the long axis of the chromophore [1, 55]. The origin of the UV–visible band of **1–6** is not entirely clear. The ~450 nm band is somewhat red-shifted from the usual dipyrrinone λ_{max} (400–420 nm) or mesobilirubin-XIIIα; the intensity is less

than that of the latter but larger than that of typical dipyrrinones [1, 3–6, 10, 46–50]. The ~450 nm band, found also in simpler 10-*nor*-rubins without alkanoic acid groups [17, 18], might be viewed as originating in conjugation rather than from exciton coupling [56]. In the simpler 10-*nor*-rubins, the bipyrrole twist angle (ϕ) is computed (PPP molecular orbital calculation) to be 65° [1, 17]. The X-ray structure indicates 38° [1]—both reveal a synclinal stereochemistry. In contrast, with limited (**1**, **3**, **5**) or full (**2**, **4**, **6**) intramolecular hydrogen bonding, the 10-*nor*-rubins are predicted to have an anticlinal stereochemisty about C(9)–C(11). Just how such a different stereochemistry may affect the UV–visible spectrum is unclear. Alternatively, one might view the broad 450 nm band(s) as having exciton coupling between the two dipyrrinones, whose UV–visible characteristics remain unknown. Irrespective of its origin, the 450 nm band in the UV–visible spectra of **1**–**6** remains essentially wavelength- and absorption-invariant over a wide range of solvent polarity (Table 4), suggesting that the relative orientation of the dipyrrinones is unchanged, as is the relative orientation of electric dipole transition moments. Further computational studies are required to sort out the origin of the 450 nm band.

Concluding comments

Unlike bilirubin, the 10-*nor*-rubins of this work cannot be folded into ridge-tile shapes such as in Fig. 1; however, with the propionic acids extended to butanoic, full intramolecular hydrogen bonding (Fig. 3) is possible and can preserve a twisted, *anticlinal*, rotated conformation about the bipyrrole core. With propionic acid chains, the lactam C=O to CO_2H hydrogen bond is longer, and the system is more polar and somewhat less conformationally stable. Interestingly, molecular modeling shows that with acetic acid chains, full hydrogen bonding (acid to dipyrrinone) is possible, but the bipyrrole is planar.

Experimental

All ^1H and ^{13}C NMR spectra were obtained on a General Electric (Fairfield, CT, USA) QE-300 or GN-300 spectrometer at 300 and 75 MHz, respectively, or on a Varian (Palo Alto, CA, USA) Unity 400 MHz spectrometer. Chemical shifts in $CDCl_3$ are reported in ppm, referenced to the residual chloroform proton signal at 7.26 ppm and its ^{13}C signal at 77.23 ppm, unless otherwise noted. All GC–MS spectra were obtained from a Varian CP-3800 mass spectrometer. Melting points were taken on a Mel-Temp apparatus. Satisfactory combustion analyses for carbon, hydrogen, and nitrogen were performed by Desert Analytics (Tucson, AZ, USA) and were within ±0.4% of theoretical values. Fast atom bombardment high-resolution mass measurements from the Nebraska Center for Mass Spectrometry gave molecular ions with exact masses within 0.7–2.8 ppm of theoretical values. Infrared spectra were recorded on a PerkinElmer (Waltham, MA, USA) Spectrum 2000 FT-IR infrared spectrophotometer. All UV–visible spectra were recorded on a PerkinElmer λ-12 spectrophotometer. Flash column chromatography was carried out using silica gel, 60–200 mesh (M. Woelm, Eschwege, Germany). Radial chromatography was carried out on 1 or 2 mm thick rotors of Merck (Darmstadt, Germany) silica gel $PF2_{54}$-gypsum, preparative layer grade, using a Chromatotron (Harrison Research, Inc., Palo Alto, CA, USA). The ball and stick drawings were created from the atomic coordinates of the molecular dynamics structures using Müller and Falk's program (Cherwell Scientific, Oxford, UK) for Macintosh.[1] Molecular dynamics calculations were carried out on a Silicon Graphics workstation using Sybyl 7.0 with a Gasteiger–Hückel calculation of electronic charge.

All solvents were reagent grade, obtained from Fisher (Pittsburgh, PA, USA) and Acros (Geel, Belgium). Deuterated chloroform, dichloromethane and dimethylsulfoxide were from Cambridge Isotope Laboratories (Andover, MA, USA); (trimethylsilyl)acetylene, propargyl alcohol, 3-butyn-1-ol and phenylacetylene were from GFS Chemicals (Powell, OH, USA). Pd(C)-10%, Cu powder (99%, for organic synthesis, batch #16920 AB), palladium acetate and $Pd(PPh_3)_2Cl_2$ were from Sigma-Aldrich (St. Louis, MO, USA. Copper(I) iodide (Cu_2I_2, stock #26110, lot #060674) was from Alfa Products (Ward Hill, MA, USA). Diethyl 3,5-dimethyl-1*H*-pyrrole-2,4-dicarboxylate (**15**) [57] and 5-bromomethylene-4-ethyl-3-methyl-2-oxo-2,5-dihydropyrrole [23], 3,4-dimethyl-3-pyrrolin-2-one [24–26] and 3,4-diethyl-3-pyrrolin-2-one [27] were prepared according to previously described procedures.

10-nor-Mesobilirubin-XIIIα (**1**, $C_{32}H_{38}N_4O_6$)
Tetrapyrrole dimethyl ester (**1e**) (30 mg, 0.05 mmol) was suspended in nitrogen-purged methanol (5 cm^3) and heated to reflux. Nitrogen-purged 1 M aqueous sodium hydroxide (2 cm^3) was then added, and reflux was continued for 6 h. The mixture was cooled to room temperature, water (5 cm^3) was added, then the methanol was removed under

[1] Molecular mechanics and dynamics calculations employed to find the global energy minimum conformation of **1** were run on an SGI Octane Workstation using version 7.0 of the Sybyl forcefield, as described in [10, 46–50]; the ball and stick drawings were created from the atomic coordinates using Müller and Falk's "Ball and Stick" program for the Macintosh (http://www.orc.uni-Linz.ac.at/mueller/ball_and_stick.shtml).

reduced pressure (rotovap). The resulting mixture was cooled to 5 °C and carefully acidified with concentrated HCl until a precipitate formed. The precipitate was collected by filtration, washed with water, and dried in vacuo to produce diacid (**1**). Yield: 17 mg (61%); mp 228–230 °C; ^{1}H NMR: δ = 1.14 (6H, t, J = 7.5 Hz), 1.84 (6H, s), 2.45 (6H, s), 2.47 (6H, t, J = 7.3 Hz), 2.50 (4H, q, J = 7.5 Hz), 2.63 (4H, t, J = 7.3 Hz), 6.34 (2H, s), 10.13 (2H, brs), 11.23 (2H, brs), 11.87 (2H, brs) ppm; ^{13}C NMR data are in Table 1; HRMS (FAB, glycerol): calcd for M + H$^+$, $C_{32}H_{39}N_4O_6$, 575.2870; found 575.2864.

10-nor-Mesobilirubin-XIIIα dimethyl ester (**1e**, $C_{34}H_{42}N_4O_6$)

To a 50 cm^3 round-bottom flask equipped with a magnetic stirrer and a heating mantle were added 3,3′-bis-(2-carboxyethyl)-4,4′-dimethyl-1*H*,1′*H*-2,2′-bipyrrole-5,5′-dicarboxylic acid (**7**) (230 mg, 0.55 mmol), 5-bromomethylene 4-ethyl-3-methyl-3-pyrrolin-2-one (250 mg, 1.16 mmol), methanol (15 cm^3), and five drops 40% aqueous HBr. The mixture was heated at reflux under a blanket of nitrogen, and after 8 h the mixture was cooled. Methanol was removed under reduced pressure (rotovap), and the dark green residue dissolved in a small portion of CH_2Cl_2. Hexane (50 cm^3) was added, and the resulting precipitate was collected by filtration. (The orange-red hexane filtrate contained primarily unreacted bromomethylenepyrrolinone.) The precipitate was redissolved in CH_2Cl_2, and the resulting solution was passed through a short column of silica gel, eluting with a mixture of CH_2Cl_2 and CH_3OH (97:3 vol/vol). The solvent was removed under reduced pressure, and tetrapyrrole dimethyl ester **1e** was purified by radial chromatography using a 97:3 vol/vol mixture of CH_2Cl_2 and CH_3OH as eluent. The purified diester was crystallized from CH_2Cl_2–hexane, collected by filtration and washed with hexane to give pure **1e**. Yield: 0.12 g (36%); mp 230–232 °C; ^{1}H NMR: δ = 1.13 (6H, t, J = 7.5 Hz), 1.82 (6H, s), 2.51 (4H, q, J = 7.5 Hz), 2.53 (3H, s), 2.62 (4H, q, J = 7.3 Hz), 10.47 (2H, brs) ppm.

3,17-Des-ethyl-3,17-dimethyl-10-nor-mesobilirubin-XIIIα (**3**, $C_{30}H_{34}N_4O_6$)

3,3′-Bis-(2-carboxyethyl)-4,4′-dimethyl-1*H*,1′*H*-2,2′-bipyrrole-5,5′-dicarboxylic acid (**7**) (30 mg, 0.71 mmol) was placed in a 100 cm^3, round-bottom flask previously cooled in an ice/water bath. TFA (0.7 cm^3) was then added, and the resulting dark brown solution was stirred in the ice/water bath for 30 min. Anhydrous triethyl orthoformate (0.27 cm^3) was added while the reaction was still being cooled. The resulting dark red solution was stirred for an additional 2 h at 0 °C, then water (50 cm^3) was added to precipitate a green solid (**9**). The solid was removed by extraction into CH_2Cl_2, and the green organic layer was washed once with 1 M aqueous NaOH solution, then with brine, before being dried (Na_2SO_4). After the solvent was removed, dialdehyde **9** was obtained as a greenish solid and used in the next step without further purification. Dialdehyde **9** and 3,4-dimethyl-3-pyrrolin-2-one [24–26] (0.31 g, 2.84 mmol) were placed in a 50 cm^3 round-bottom flask containing a magnetic stir bar. To the mixture of solids was added 3 cm^3 of methanol and 10 cm^3 of 4 M aqueous KOH, and the solution was stirred at room temperature for 12 h, then warmed briefly (over 30 min) almost to reflux. The cooled solution was then acidified to pH 3 with concentrated HCl. A bright yellow solid (**3**) was separated, repeatedly washed with water, collected by centrifugation and dried. Yield: 0.15 g (38% from **9**); mp 232–235 °C; ^{1}H NMR: δ = 1.77 (6H, s), 2.25 (6H, s), 2.45 (6H, s), 2.47 (4H, t, J = 7.4 Hz), 2.63 (4H, t, J = 7.4 Hz), 6.68 (2H, s), 10.11 (2H, brs), 11.15 (2H, brs), 11.90 (2H, brs) ppm; ^{13}C NMR data are in Table 1.

2,18-Des-methyl-2,18-diethyl-10-nor-mesobilirubin-XIIIα (**5**, $C_{34}H_{42}N_4O_6$)

Dialdehyde **9**, prepared from 3,3′-bis-(2-carboxyethyl)-4,4′-dimethyl-1*H*,1′*H*-2,2′-bipyrrole-5,5′-dicarboxylic acid (**7**) (30 mg, 0.71 mmol) as described above, and 3,4-diethyl-1*H*-pyrrolin-2-one [27] (0.39 g, 2.84 mmol) were treated as above for the synthesis of **5**. The desired yellow solid diacid (**5**) was isolated as above. Yield: 0.17 g, 40% (from **7**); mp 135–138 °C; ^{1}H NMR: δ = 1.11 (6H, t, J = 7.5 Hz), 1.12 (6H, t, J = 7.4 Hz), 2.25 (4H, q, J = 7.5 Hz), 2.40 (4H, q, J = 7.4 Hz), 2.45 (6H, s), 2.47 (4H, t, J = 7.5 Hz), 2.63 (4H, t, J = 7.5 Hz), 6.49 (2H, s), 10.39 (2H, brs), 11.29 (2H, brs), 11.81 (2H, brs) ppm; ^{13}C NMR data are in Table 1.

8,12-Des-propionic acid-8,12-bis-butyric acid-10-nor-mesobilirubin-XIIIα (**2**, $C_{34}H_{42}N_4O_6$)

Using the procedure for the preparation of **1** from **1e**, tetrapyrrole dimethyl ester **2e** (82 mg, 0.13 mmol) was suspended in nitrogen-purged methanol (4 cm^3) and heated to reflux. Nitrogen-purged aqueous sodium hydroxide (1.5 cm^3, 1 M) was then added, and reflux was continued for 6 h. Work-up as for **1** afforded diacid **2**. Yield: 38 mg (51%); mp 153–156 °C; ^{1}H NMR: δ = 1.13 (6H, t, J = 7.5 Hz), 1.70 (4H, m), 1.82 (6H, s), 2.36 (4H, t, J = 7.4 Hz), 2.51 (8H, m), 2.63 (6H, s), 6.12 (2H, s), 9.30 (2H, brs), 10.77 (2H, brs), 13.35 (2H, brs) ppm; ^{13}C NMR: δ = 8.9 (q), 10.3 (q), 15.2 (q), 20.0 (t), 22.0 (t), 24.0 (t), 34.0 (t), 99.6 (d), 124.6 (s), 125.4 (s), 130.0 (s), 130.5 (s), 130.8 (s), 132.1 (s), 137.7 (s), 173.6 (s), 177.9 (s) ppm; ^{13}C NMR data in $(CD_3)_2SO$ are in Table 1.

8,12-Des-(2-methoxycarbonylethyl)-8,12-(3-carboxymethyl)propyl-10-nor-mesobilirubin-XIIIα (**2e**, $C_{36}H_{40}N_4O_6$)

Following the procedure for the synthesis of **1e**, 3,3′-bis-(3-carboxypropyl)-4,4′-dimethyl-1*H*,1′*H*-2,2′-bipyrrole-5,5′-

dicarboxylic acid **17** (210 mg, 0.5 mmol) and 5-bromomethylene-4-ethyl-3-methyl-3-pyrrolin-2-one (237 mg, 1.1 mmol) in 12 cm^3 methanol with four drops aqueous HBr (40%) gave dimethyl ester **2e**. Yield: 82 mg (26%); mp 154–155 °C; ^{1}H NMR: δ = 1.13 (6H, t, J = 7.5 Hz), 1.82 (10H, m), 2.30 (4H, t, J = 7.4 Hz), 2.51 (4H, q, J = 7.5 Hz), 2.63 (10H, m), 3.60 (6H, s), 6.73 (2H, s), 10.9 (2H, brs), 10.41 (2H, brs); ^{13}C NMR: δ = 8.5 (q), 10.8 (q), 15.0 (q), 17.2 (t), 25.6 (t), 26.2 (t), 33.2 (t), 51.6 (q), 98.1 (d), 121.3 (s), 123.4 (s), 124.8 (s), 127.3 (s), 130.3 (s), 134.6 (s), 149.4 (s), 172.4 (s), 172.8 (s) ppm.

3,17-Des-ethyl-3,17-dimethyl-8,12-des-propionic acid-8,12-bis-butyric acid-10-nor-mesobilirubin-XIIIα (**4**, $C_{32}H_{38}N_4O_6$)

Using the procedure for the synthesis of **3**, 3,3′-bis-(3-carboxypropyl)-4,4′-dimethyl-1*H*,1′*H*-2,2′-bipyrrole-5,5′-dicarboxylic acid (**17**) (596 mg, 1.42 mmol) was placed in a 100 cm^3 round-bottom flask previously cooled in an ice/water bath, TFA (1.4 cm^3) was then added, and the resulting dark brown solution stirred in the ice/water bath for 30 min. Anhydrous triethyl orthoformate (0.52 cm^3) was added while the reaction was still being cooled, and the resulting dark red solution of aldehyde **18** was treated as in **3**. 3,4-Dimethyl-3-pyrrolin-2-one [24–26] (790 mg, 7.1 mmol) was used in the NaOH-catalyzed condensation to afford **4**. Yield: 237 mg (29% from **24**); mp 142–144 °C; ^{1}H NMR: δ = 1.73 (10H, m), 2.25 (6H, s), 2.33 (6H, t, J = 7.5 Hz), 2.64 (6H, t, J = 7.4 Hz), 6.60 (2H, s), 9.22 (2H, brs), 10.75 (2H, brs), 13.44 (2H, brs) ppm; ^{13}C NMR: δ = 9.0 (q), 10.5 (q), 20.1 (t), 22.1 (t), 24.2 (q), 34.3 (t), 99.7 (d), 124.3 (s), 124.8 (s), 125.5 (s), 130.2 (s), 130.5 (s), 130.9 (s), 132.1 (s), 137.7 (s), 173.8 (s), 178.0 (s) ppm; ^{13}C NMR data in $(CD_3)_2SO$ are in Table 1.

2,18-Des-methyl-2,18-diethyl-8,12-des-propionic acid-8,12-bis-butyric acid-10-nor-mesobilirubin-XIIIα (**6**, $C_{36}H_{48}N_4O_6$)

As in the synthesis of **5**, 3,3′-bis-(3-carboxypropyl)-4,4′-dimethyl-1*H*,1′*H*-2,2′-bipyrrole-5,5′-dicarboxylic acid (**17**) (25 mg, 0.6 mmol), TFA (0.7 cm^3) and anhydrous triethyl orthoformate (0.25 cm^3) were used to generate aldehyde **18**. Upon treatment with 3,4-diethyl-1*H*-pyrrolin-2-one [27] (417 mg, 3 mmol) in an NaOH-catalyzed condensation, the desired diacid **6** was obtained. Yield: 117 mg (31% from **24**); mp 146–148 °C; ^{1}H NMR: δ = 1.10 (12H, m), 1.70 (4H, m), 2.25 (4H, q, J = 7.5 Hz), 2.33 (4H, t, J = 7.6 Hz), 2.40 (4H, q, J = 7.5 Hz), 2.69 (10H, m), 6.32 (2H, s), 9.44 (2H, brs), 10.69 (2H, brs), 13.60 (2H, brs) ppm; ^{13}C NMR: δ = 9.0 (q), 10.4 (s), 14.1 (q), 15.5 (q), 20.2 (t), 22.1 (t), 22.1 (t), 34.3 (t), 100.0 (d), 124.0 (s), 124.7 (s), 125.5 (s), 130.2 (s), 130.6 (s), 130.8 (s), 132.2 (s), 137.7 (s), 173.8 (s), 176.9 (s) ppm; ^{13}C NMR data in $(CD_3)_2SO$ are in Table 1.

3,3′-Bis-(2-carboxyethyl)-4,4′-dimethyl-1H,1′H-2,2′-bipyrrole-5,5′-dicarboxylic acid (**7**, $C_{18}H_{20}N_2O_8$)

A mixture of tetraester **8** (1.00 g, 1.97 mmol), NaOH (0.67 g, 0.017 mol), 95% ethanol (9 cm^3) and H_2O (3 cm^3) was heated at reflux for 3 h. The ethanol was removed by distillation, and the residue was cooled. Then a solution of $NaNO_2$ (6.5 g, 0.076 mol) in water (10 cm^3) was added, and the flask was cooled to −10 °C in a dry ice/acetone bath. Concentrated HNO_3 (1.5 cm^3) (at −20 °C) was added very slowly to keep the temperature at −10 °C, and the mixture was stirred an additional 30 min after the addition was complete. The colorless product was collected by filtration, washed with cold H_2O and dried at 0.7 mm Hg in a dessicator over P_2O_5 overnight to afford a pink solid. Yield: 0.69 g (92%); ^{13}C NMR ($(CD_3)_2SO$): δ = 10.5, 19.6, 34.0, 119.3, 122.0, 124.4, 125.2, 162.4, 174.8 ppm; HRMS (FAB, glycerol): calcd for M + H^+, $C_{22}H_{28}N_2O_8$, 393.1298; found 393.1317.

Diethyl 3,3′-bis-(2-ethoxycarbonylethyl)-4,4′-dimethyl-2,2′-bipyrrole-5,5′ dicarboxylate (**8**, $C_{26}H_{36}N_2O_8$)

Diethyl 3,3′-bis-(2-ethoxycarbonylethenyl)-4,4′-dimethyl-2,2′-bipyrrole-5,5′-dicarboxylate (**10**) (40 mg, 0.072 mmol) was dissolved in 12 cm^3 absol. ethanol and hydrogenated at room temperature and atmospheric pressure over 10% PtO_2 (1.6 mg, 0.0072 mmol) for 1.5 h. After filtration through a pad of silica gel, the solution was evaporated to yield the reduced product (**8**), which was purified by radial chromatography (CH_2Cl_2 eluent). Yield: 0.037 g (92%); mp 151–152 °C; ^{1}H NMR: δ = 1.29 (6H, t, J = 7.4 Hz), 2.26 (6H, s), 2.40 (2H, t, J = 7.5 Hz), 2.70 (2H, t, J = 7.5 Hz), 4.14 (4H, q, J = 7.5 Hz), 4.10 (4H, q, J = 7.4 Hz), 9.0 (2H, brs) ppm; HRMS (FAB, glycerol): calcd for M + H^+, $C_{26}H_{37}N_2O_8$, 505.2549; found 505.2553.

Diethyl 3,3′-bis-(2-ethoxycarbonylethenyl)-4,4′-dimethyl-2,2′-bipyrrole-5,5′-dicarboxylate (**10**, $C_{26}H_{36}N_2O_8$)

A mixture of ethyl 4,5-diiodo-3-methyl-1*H*-pyrrole-2-carboyxlate (**11**) (0.37 g, 0.9 mmol), freshly distilled triethyl amine (0.25 cm^3, 1.8 mmol), ethyl acrylate (0.2 cm^3, 1.8 mmol), $PdCl_2(PPh_3)_2$ (0.014 g, 0.02 mmol), and acetonitrile (50 cm^3) was heated to 60 °C and stirred under N_2 for 12 h. The reaction mixture was cooled to room temperature and taken up in CH_2Cl_2 (25 cm^3). The solution was washed with cold dilute 0.5 M aqueous HCl, saturated NaCl, and dried ($MgSO_4$). Evaporation of the solvent (rotovap) gave the crude product, which was purified by radial chromatography (hexane/Et_2O, 4:1, vol/vol). Yield: 0.26 g (58%); mp 140–141 °C; ^{13}C NMR: δ = 14.3, 14.5, 27.2, 60.5, 60.9, 118.0, 120.5, 121.7, 126.3, 133.2, 136.2, 161.5, 167.7 ppm; HRMS (FAB, 3-NBA): calcd for $C_{26}H_{36}N_2O_8$, 504.2472; found 504.2491.

Ethyl 4,5-diiodo-3-methyl-1H-pyrrole-2-carboxylate (**11**, $C_8H_9I_2NO_2$)

In a 1,000 cm^3 beaker, 10 g (41.46 mmol) of crude 5-(ethoxycarbonyl)-4-methyl-1*H*-pyrrole-2,3-dicarboxylic acid (**12**) was dissolved in 70 cm^3 of hot ethanol. Sodium bicarbonate (17.8 g, 207.3 mmol) in 100 cm^3 of water was added carefully, in portions, to the mixture. The mixture was then heated to 65 °C on a hot plate. Potassium iodide (40.4 g, 240 mmol) and iodine (13.16 g, 103.7 mmol) were dissolved in 350 cm^3 of water at 50–55 °C, and the mixture was then added dropwise, as quickly as it was decolorized in order to minimize the gas evolution. The endpoint was colored slightly purple. The contents of the flask were cooled to 50 °C, and precipitated crude **11** was collected by vacuum filtration. Solid **11** was dissolved in CH_2Cl_2 and dried over sodium sulfate. Sodium sulfate was removed by gravity filtration, and the solvent was removed under reduced pressure (rotovap). The product was crystallized from 2-propanol:water (50/50, vol/vol) to give **11** as a gray solid. Yield: 14.8 g (65% from **15**); mp 141–142 °C; ^{13}C NMR ($(CD_3)_2SO$): δ = 14.4 (q), 15.6 (q), 61.0 (t), 81.9 (s), 84.9 (s), 124.8 (s), 132.3 (s), 160.2 (s) ppm.

5-(Ethoxycarbonyl)-4-methyl-1H-pyrrole-2,3-dicarboxylic acid (**12**, $C_{10}H_{11}NO_6$)

3,5-Bis-(ethoxycarbonyl)-4-methyl-1*H*-pyrrole-2-carboxylic acid, **13** [23] (9.9 g, 37.14 mmol) was carefully added in portions to 100 cm^3 of concentrated sulfuric acid at 100 °C and stirred for 20 min. The solution was then poured into 700 cm^3 of ice and water and stirred vigorously for 10 min. The precipitated crude product, known since 1925 [58], was then collected by vacuum filtration and used directly in the preparation of **11**. ^{13}C NMR ($(CD_3)_2SO$): δ = 10.78 (q), 13.9 (q), 60.6 (t), 118.8 (s), 122.2 (s), 126.9 (s), 127.0 (s), 160.8 (s), 161.6 (s), 165.1 (s) ppm.

3,5-Bis-(ethoxycarbonyl)-4-methyl-1H-pyrrole-2-carboxylic acid (**13**, $C_{12}H_{15}NO_6$)

2,4-Diethyl-3,5-dimethyl-1*H*-pyrrole-2,4-dicarboxylate (**15**) (15.8 g, 66.3 mmol) was added to 150 cm^3 of glacial acetic acid in a three-neck round-bottom flask. The contents were stirred mechanically while being cooled to 10 °C in an ice bath. Bromine (3.5 cm^3) was added to the solution all at once, then freshly distilled sulfuryl chloride (16 cm^3) was added dropwise over 1.5 h. During the addition, care was taken to ensure that the mixture was stirred vigorously (the contents of the flask will precipitate massively during addition). After all of the sulfuryl chloride was added, the solution was allowed to reach room temperature while being stirred for 4.5 h. Water (40 cm^3) was then added dropwise, and after the addition was complete, the solution was heated on a hot water bath at 60 °C for 30 min. The solution was then poured into 1,000 cm^3 ice and water with stirring. The precipitate was collected by vacuum filtration and washed with water. The precipitate was dissolved in saturated sodium bicarbonate. The undissolved aldehyde was collected by vacuum filtration and the filtrate was acidified with dilute HCl. The resulting precipitate was collected by vacuum filtration, washed with water, and dried. Acid **13** was recrystallized from 2-propanol to afford 9.23 g. Aldehyde **14** (the side product of this reaction) was dissolved in 150 cm^3 of reagent grade acetone, and a solution of $KMnO_4$ [5 g in 200 cm^3 of acetone–water (1:1 by volume)] was added over 1 h. After 10 h of stirring at room temperature, the purple solution was poured into 250 cm^3 of a solution of 10% $NaHSO_3$, the precipitate was removed by vacuum filtration, and the yellow aqueous solution was acidified with dilute HCl. The resulting white precipitate was collected by vacuum filtration, washed with water, and dried. Acid **13** was recrystallized from 2-propanol. Yield: 11.2 g (70% combined yield); mp 271–272 °C ([57], mp 273–274 °C); ^{13}C NMR ($(CD_3)_2SO$): δ = 10.7 (q), 13.9 (q), 14.1 (q), 60.3 (t), 60.6 (t), 118.9 (s), 121.4 (s), 127.3 (s), 127.5 (s), 160.2 (s), 160.8 (s), 165.0 (s) ppm.

3,3′-Bis-(3-carboxypropyl)-4,4′-dimethyl-1H,1′H-2,2′-bipyrrole-5,5′-dicarboxylic acid (**17**, $C_{20}H_{24}N_2O_8$)

A mixture of dimethyl ester **19** (0.99 g, 1.97 mmol), NaOH (0.67 g, 0.017 mol), 95% ethanol (9 cm^3) and H_2O (3 cm^3) was heated at reflux for 3 h, after which the ethanol was removed by distillation. To the cooled residue was added 10 cm^3 of an aqueous solution of $NaNO_3$ (6.5 g, 0.076 mol), and the contents were cooled at −10 °C in a dry ice/acetone bath. Concentrated HNO_3 (1.5 cm^3) (cooled to −20 °C) was added very slowly, with stirring, to keep the temperature at −10 °C, and the mixture was stirred an additional 30 min after the addition was complete. The precipitate was collected by filtration, washed with cold H_2O and dried at 0.7 mm Hg in a dessicator over P_2O_5 overnight to afford a pink solid. Yield: 0.75 g (91%); mp 185.6 °C; ^{13}C NMR ($(CD_3)_2SO$): δ = 11.5, 19.6, 20.3, 35.0, 118.3, 121.8, 124.2, 124.6, 162.4, 174.9 ppm; HRMS (FAB, glycerol): calcd for M + H^+, $C_{20}H_{25}N_2O_8$, 421.1611; found 421.1623.

Diethyl 3,3′-bis-(3-methoxycarbonyl-1-propyl)-4,4′-dimethyl-2,2′-1H,1′H-bipyrrole-5,5′-dicarboxylate (**19**, $C_{26}H_{36}N_2O_8$)

Diethyl 3,3′-bis-(3-methoxycarbonyl-1-propynyl)-4,4′-dimethyl-2,2′-1*H*,1′*H*-bipyrrole-5,5′-dicarboxylate (**20**) (0.36 g, 0.72 mmol) was dissolved in 12 cm^3 methanol and hydrogenated at room temperature and atmospheric pressure over 10% PtO_2 (0.016 g, 0.072 mmol) for 1.5 h. The resulting solution was vacuum-filtered through a pad of silica gel, and the solvent was evaporated to give crude **19**, which was purified by radial chromatography (CH_2Cl_2, eluent). Yield: 0.31 g (85%); mp 158–159 °C; ^{1}H NMR: δ

= 1.35 (6H, t, J = 7.5 Hz), 1.82 (4H, m), 2.29 (10H, m), 2.70 (4H, m), 3.56 (6H, s), 4.28 (4H, t, J = 7.6 Hz), 8.50 (2H, brs) ppm; HRMS (FAB, 3-NBA): calcd for $C_{26}H_{36}N_2O_8$, 504.2472; found 504.2482.

Diethyl 3,3′-bis-(3-methoxycarbonyl-1-propynyl)-4, 4′-dimethyl-2,2′-1H,1′H-bipyrrole-5,5′-dicarboxylate (**20**, $C_{26}H_{28}N_2O_8$)

Ethyl 4,5-diiodo-3-methyl-1*H*-pyrrole-2-carboxylate, **11** (0.28 g, 0.7 mmol), $PdCl_2(PPh_3)_2$ (0.05 g, 0.07 mmol), CuI (0.03 g, 0.14 mmol) and methyl 3-butynoate [56] (0.27 g, 2.8 mmol) were dissolved in 15 cm^3 freshly distilled triethyl amine. The mixture was heated at 60 °C and stirred under nitrogen overnight. After cooling, the solvent was evaporated at reduced pressure, and the crude product was redissolved in CH_2Cl_2 and vacuum filtered through a pad of silica gel. The CH_2Cl_2 was evaporated (rotovap) to yield crude **20**, which was purified by radial chromatography (CH_2Cl_2, eluent). Yield: 0.24 g (68%); mp 154–155 °C; ^{1}H NMR: δ = 1.35 (6H, t, J = 7.6 Hz), 2.45 (6H, s), 3.67 (10H, m), 4.28 (8H, m), 9.38 (2H, brs) ppm. The compound was hydrogenated directly to **19**.

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