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Enantioselection in bilirubin analogs with only one propionic acid group

Stefan E. Boiadjiev and David A. Lightner *
Department of Chemistry, University of Nevada, Reno, Nevada 89557-0020, USA

Abstract: Enantiopure synthetic bilirubin analogs (**1** and **2**) with only a single β-methyl propionic acid group adopt a folded, ridge-tile conformation stabilized by intramolecular hydrogen bonding. The β-methyl group forces the pigment to adopt a left-handed (*M*) helical conformation, as evidenced by exciton circular dichroism spectra and indicating that one propionic acid group is sufficient to control the pigment's conformation. © 1997 Elsevier Science Ltd

Introduction

Bilirubin (Figure 1), the yellow–orange neurotoxic pigment of jaundice, is a tetrapyrrole dicarboxylic acid formed in the normal metabolism of heme proteins.[1] In a healthy adult, it is produced at the rate of ~300 mg/day, principally from the breakdown of red blood cells. Bilirubin is a conformationally mobile bichromophore with characteristics of a molecular propeller.

Rotation of its two dipyrrinone chromophores about the central C(10) CH_2 unit generates a large number of conformational isomers, of which folded conformations, shaped like a ridge-tile have minimized non-bonded steric interactions.[2,3] The ridge-tile conformation, which is not rigid, brings the pigment's propionic acid groups into close proximity of the dipyrrinone NH and C=O groups, thus easily engaging a network of six intramolecular hydrogen bonds to make the ridge-tile conformation unusually stable.[2] This inward tucking of the CO_2H groups and tethering to opposing dipyrrinones through intramolecular hydrogen bonding decreases the polarity of the pigment, leaving it unexcretable in normal metabolism (hepatic excretion), except by glucuronidation.[1,4] The ridge-tile conformation is found in crystalline bilirubin and its salts,[5,6] and it is the favored conformation in solution.[7,8] However, when the propionic acid groups are translocated away from C(8) and C(12) [e.g., to C(7) and C(13)] the solution properties of the pigment undergo significant changes.[9] Such pigments are less lipophilic than bilirubin and much less soluble in non-polar organic solvents. They are also typically excretable (hepatic excretion) without glucuronidation.[10] However, analogs with propionic acid groups at C(8) and C(12), such as mesobilirubin-XIIIα, typically share bilirubin's unique lipophilic and hepatic excretion, because they also have their CO_2H groups sequestered by intramolecular hydrogen bonding (Figure 1).

Bilirubin intramolecular hydrogen bonding (Figure 1) is one of the most interesting and important facets of bilirubin conformation.[2–8] Although the two component dipyrrinone units of bilirubin-type molecules may rotate relatively freely and independently about the interconnecting central CH_2 group, two non-superimposable mirror image conformations are uniquely stabilized through an extensive network of intramolecular hydrogen bonds. These conformational enantiomers are known to interconvert fairly rapidly at room temperature over a barrier of ~20 kcal/mole.[7] Our interest in stabilization of pigment stereochemistry through the action of intramolecular hydrogen bonding led us to consider whether such hydrogen bonding might be retained in a bilirubin analog with only one propionic acid group.

* Corresponding author.

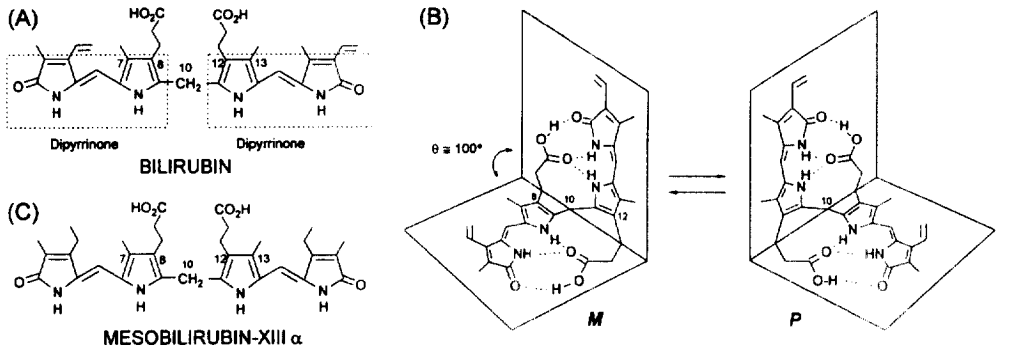
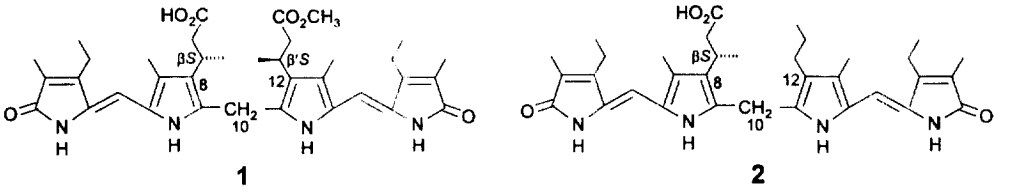


Figure 1. (A) Linear conformation of bilirubin; (B) interconverting, enantiomeric ridge-tile conformations of bilirubin stabilized by intramolecular hydrogen bonding; (C) linear conformation of mesobilirubin-XIIIα.

For this study we chose two mesobilirubin-XIIIα models with only one propionic acid and stereogenic centers in the propionate groups due to placement of one methyl group at the β-position of the propionic acid side chains; **1**, the mono-acid ester of the previously described diacid,[8a] and **2**, a mono-acid with one propionic acid at C(8) and an *n*-propyl group at C(12). The stereogenic centers allowed us to prepare optically pure isomers with β(*S*) stereochemistry for use in probing pigment conformation through circular dichroism (CD) spectroscopy. As will be illustrated, the β-methyl groups perturb the equilibrium depicted in Figure 1 through non-bonded steric interactions with the C(10) CH_2 group that shift the equilibrium toward the *M*-chirality conformer.[2] Such an equilibrium displacement, however, can occur only in the intramolecularly hydrogen-bonded conformers; thus, these optically active pigments can be viewed as useful chiral probes of pigment stereochemistry and intramolecular hydrogen bonding. In the following we report on the synthesis, spectroscopic properties and conformational analysis of **1** and **2**.

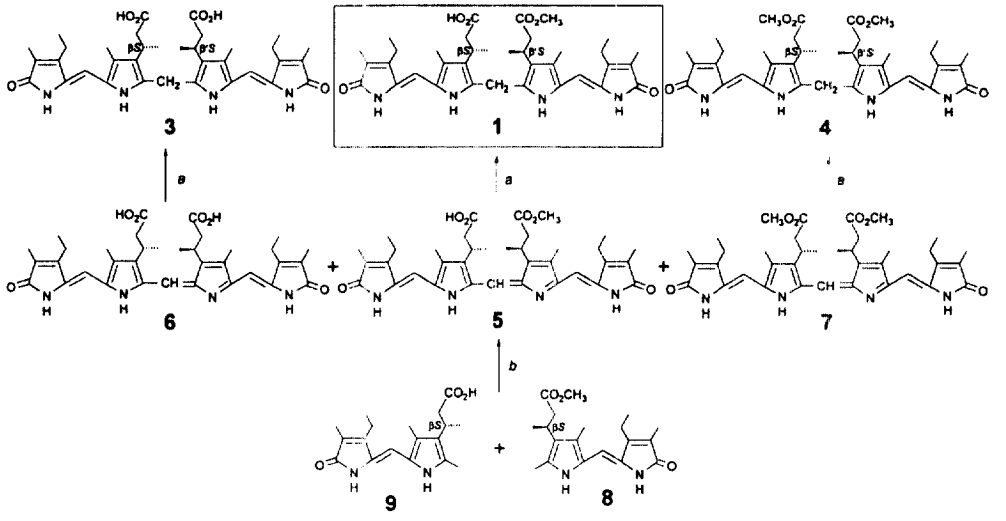


Results and discussion

Synthesis

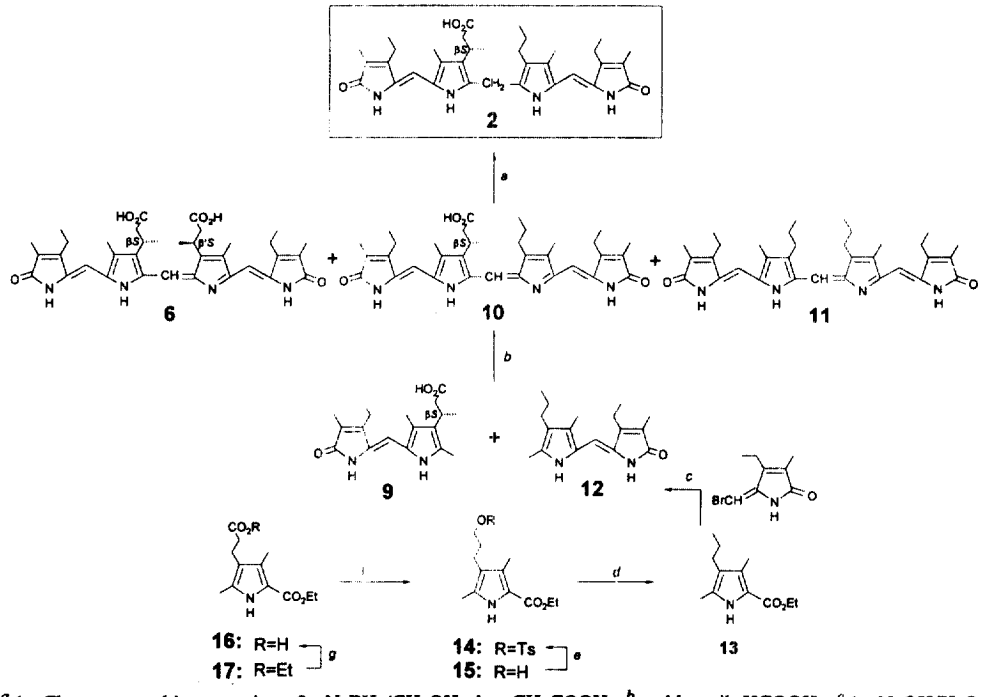
The target mono-acids (**1** and **2**) were obtained through the series of steps outlined in Schemes 1 and 2. The verdin mono-acid **5** was prepared from the known dipyrrinone acid (**9**)[11] and its methyl ester (**8**)[8a] by oxidative cross-coupling using *p*-chloranil (Scheme 1), and the resulting mixture of verdin diacid (**6**), diester (**7**) and mono-acid (**5**) was separated by radial chromatography, collecting the bright blue band of intermediate polarity to afford a 69% yield of **5**. Reduction of **5** with sodium borohydride in THF–MeOH afforded a 67% yield of the bright yellow mono-acid **1**. Collection of separated **6** and **7**, followed by sodium borohydride reduction afforded rubin diacid **3** and its dimethyl ester (**4**).

Synthesis of mono-acid **2** was also accomplished by oxidative cross-coupling of two different dipyrrinones, **9** and **12** to give a mixture of verdins (**6**, **10** and **11**, Scheme 2). Separation of this intensely blue mixture by radial chromatography afforded a 62% yield of **10**, which was reduced using $NaBH_4$ to give bright yellow rubin mono-acid **2** in 54% yield. Although dipyrrinone **9** was known from earlier work,[11] dipyrrinone **12** was not. It was prepared from the known monopyrrole diester **17**[12] in a series of steps that transformed its propionic ester group to *n*-propyl. Selective saponification



[a] 1. Chromatographic separation; 2. $NaBH_4/CH_3OH$, then CH_3COOH. [b] *p*-chloranil, HCOOH.

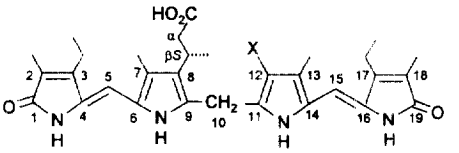
Scheme 1.



[a] 1. Chromatographic separation; 2. $NaBH_4/CH_3OH$, then CH_3COOH. [b] *p*-chloranil, HCOOH. [c] 1. $NaOH/H_2O$, then HNO_3; 2. 5-bromomethylene-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole/C_2H_5OH. [d] NaI, Zn. [e] TsCl, Et_3N. [f] BH_3. THF. [g] $NaOH/H_2O$, then HCl.

Scheme 2.

Table 1. Comparison of ^{13}C-NMR data for mesobilirubin monoacids **1** and **2** with diacid **3** and dimethyl ester **4** in $CDCl_3$ at 22°C[a]



1: X=(*S*)-CH(CH$_3$)CH$_2$CO$_2$CH$_3$
2: X=CH$_2$CH$_2$CH$_3$
3: X=(*S*)-CH(CH$_3$)CH$_2$CO$_2$H
4: Dimethyl ester of **3**

Position	1	2	3	4
1,19-CONH	172.62, 174.94	173.83, 175.02	174.92	173.50
2,18	123.44, 124.17	124.00, 124.05	123.35	123.57
2,18-CH$_3$	7.96, 8.27	7.96, 8.46	7.95	7.68
3,17	147.58, 148.29	148.17, 148.29	148.39	147.07
3,17-CH$_2$CH$_3$	17.81, 17.88	17.84, 17.91	17.82	17.77
3,17-CH$_2$CH$_3$	14.75, 14.87	14.77, 14.89	14.90	14.72
4,16	128.31, 129.40	128.13, 129.89	128.25	128.85
5,15-CH=	97.49, 100.25	99.32, 101.38	100.29	99.42
6,14	122.56, 123.39	123.10, 123.51	123.31	122.77
7,13	120.56, 122.21	121.38, 122.02	122.42	121.71
7,13-CH$_3$	10.92, 11.10	9.67, 11.15	11.01	10.96
8,12	124.62, 124.80	124.64, 124.95	124.84	124.30
β,β′-CH	26.68, 27.20	27.09, 25.97[b]	26.47	27.93
β,β′-CH$_3$	21.10, 21.26	21.31	20.94	20.72
α,α′-CH$_2$	39.51, 40.24	24.46, 39.52	39.56	41.30
α,α′-COOR	175.60, 179.36	179.12, 13.99[c]	180.14	173.92
9,11	130.64, 132.70	131.01, 132.10	132.93	130.50
10-CH$_2$	21.50	22.28	21.85	22.73
α,α′-COOCH$_3$	52.74	—	—	51.58

[a] Values reported for 10^{-2} M solutions in ppm downfield from $(CH_3)_4Si$. [b] β′-CH$_2$. [c] α′-CH$_3$.

afforded a 93% yield of mono-acid **16**, whose acid group was reduced selectively using diborane to afford hydroxypropylpyrrole ester **15** in 95% yield. Formation of the *p*-toluenesulfonate ester in 83% yield followed by treatment with sodium iodide and zinc at reflux in glyme–water gave a 97% yield of **13**, which was saponified and coupled with 5-bromomethylenepyrrolinone[12a,c] to afford a 72% yield of bright yellow dipyrrinone **12**.

Structure and ^{13}C-NMR spectra

The ^{13}C-NMR for rubin mono-acids **1** and **2** are consistent with the assigned structures and may be compared (Table 1) to the similar data of diacid **3** and diester **4**. Not surprisingly, the ^{13}C-NMR spectrum of **1** shows doubling of most signals and is rather similar to a composite of the spectrum of **3** and of **4**. The ^{13}C-NMR spectrum of **2** also shows signal doubling, as may also be expected from its non-symmetric structure.

^{1}H-NMR analysis and intramolecular hydrogen bonding

Intramolecular hydrogen bonding is believed to be the most important factor in stabilizing the folded, ridge-tile conformation of bilirubin in the crystal[3,5] and in solution in non-polar solvents.[3,7]

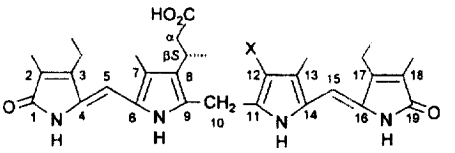
It is thought to be important even in polar and hydroxylic solvents,[3,7,8] and it is of great importance to the success of the allosteric model essential to this work. The ^{1}H-NMR data (Table 2) for mono-acids **1** and **2** may be compared with those of diacid **3** and diester **4**. Again signal doubling is seen in **1** and **2**, which are less symmetric than **3** and **4**. Significantly, the proton chemical shifts are very similar. Of special importance are the lactam and pyrrole N*H* chemical shifts in $CDCl_3$, a solvent in which intramolecular hydrogen bonding has been well established for bilirubin.[3,7,8] In **1–3** the strongly deshielded lactam N*H* signals suggest the presence of hydrogen bonding to the propionic carbonyl, and the relatively more shielded pyrrole N*H* resonances are a strong indication of a folded conformation, as depicted in Figure 1B, where one pyrrole N*H* lies above the π-system of the other. The strongly deshielded acid O*H* confirms its involvement in hydrogen bonding (to the lactam C=O). One set of pyrrole and lactam NH chemical shifts (~9 and ~10.8 ppm, respectively) of **1** and **2** are indicative of a strongly hydrogen bonded propionic acid group and a ridge-tile conformation. The remaining sets (pyrrole NH ~8.4 ppm and lactam NH 8.7–9.1 ppm) are rather similar, suggesting that the ester carbonyl in **1** is poorly hydrogen bonded. The data may be contrasted with differing data for **4**, which (while it may adopt a folded conformation) is thought to be *inter*molecularly hydrogen bonded.

Further support for a stable, intramolecularly hydrogen-bonded ridge-tile conformation in **1** and **2** comes from analysis of vicinal HlH coupling in the propionic acid side chains (Table 2). Confirming a molecular shape where the propionic residues are constrained to adopt fixed conformations, vicinal HlH coupling constants (**1**: $^3J_{AX}$=2.7 Hz, $^3J_{BX}$=12.2 Hz; **2**: $^3J_{AX}$=3.2 Hz, $^3J_{BX}$=12.3 Hz; and, for comparison, **3**: $^3J_{AX}$=3.0 Hz, $^3J_{BX}$=12.3 Hz) in the propionic acid segment [$CH_X(CH_3)$–CH_AH_B–CO_2H] are not the averaged values seen in flexible propionic acid chains of the pigments in $(CD_3)_2SO$: $^3J_{AX}$=$^3J_{BX}$≃8 Hz.[8a] The data are consistent with a fixed and staggered geometry in the propionic acid, with an H_X–C–C–H_A torsion angle of ~60° and H_X–C–C–H_B torsion angle of ~180°—close to that seen in Dreiding or CPK models of the hydrogen-bonded pigments or in their global energy minimum conformations.[8a] It is important to note that ^{1}H-NMR ABX coupling patterns indicate that the inversion between conformational enantiomers depicted in Figure 1B is slow on the NMR time scale for **1** and **2** as well as **3**. Even the propionate ester segment of **1** displays an ABX pattern with dissimilar constants ($^3J_{AX}$=4.5 Hz, $^3J_{BX}$=12.2 Hz), rather like those of the propionic acid segment. These data suggest that in **1**, the propionate ester group, like the propionic acid, is also tied to its opposing dipyrrinone by intramolecular hydrogen bonding, thus restricting segmental motion in these chains to a fixed staggered conformation. However, in diester **4**, the vicinal coupling constants found ($^3J_{AX}$=6.7 Hz and $^3J_{BX}$=9.2 Hz) differ from pigments with one or two propionic acids (**1–3**). They reflect greater segmental motion in the propionate chains consistent with a structure with only weak intramolecular hydrogen bonding.

Conformational analysis

In **1**, **2** and **3** the ^{1}H-NMR data clearly indicate a predominance of intramolecularly hydrogen-bonded ridge-tile structures such as those in Figure 1B. Intramolecular allosteric effects of the β(*S*)-methyl groups substituted on the propionic side chains of such ridge-tile structures are predicted to displace the conformational equilibrium toward the ***M*** isomer.[2,8] Inspection of the steric environment of each of the diastereotopic hydrogens in the $CH(CH_3)$–CH_2 fragment of the intramolecularly hydrogen-bonded propionic acid groups shows that the ***M*** ⇆ ***P*** equilibrium of Figure 1 is displaced by non-bonded steric interactions. When folded into the ***M***-chirality ridge-tile enantiomer, a β(*R*)-methyl, but not a β(*S*), is brought into close non-bonded contact with the central CH_2 group at C(10). On the other hand, in the ***P***-chirality enantiomer, it is the β(*S*)-methyl hydrogens that are buttressed against the C(10) CH_2 group. Consequently, when **1**, **2** or **3** adopts either of the thermodynamically preferred intramolecularly hydrogen bonded ridge-tile conformations, one conformational enantiomer is expected to be destabilized relative to the other through allosteric action. Earlier we showed that a β(*S*) methyl group in each propionic acid chain (as in **3** or **4**) shifts the conformational equilibrium toward ***M*** by destabilizing the ***P***-chirality intramolecularly hydrogen-bonded conformational enantiomer through the introduction of a severe non-bonded β-CH_3/C(10) CH_2

Table 2. Comparison of ^{1}H-NMR data for mesobilirubin monoacids **1** and **2** with diacid **3** and dimethyl ester **4** in 10^{-3} M $CDCl_3$ solutions at 22°C



1: X=(*S*)-CH(CH$_3$)CH$_2$CO$_2$CH$_3$
2: X=CH$_2$CH$_2$CH$_3$
3: X=(*S*)-CH(CH$_3$)CH$_2$CO$_2$H
4: Dimethyl ester of **3**

Proton	1	2	3	4
21,24-NHCO	9.12 10.85	8.74 10.86	10.68	10.06
22,23-NH	8.37 9.14	8.43 8.97	9.04	9.93
α,α′-COO*H*	13.6	13.7	13.6	—
α,α′-COOC*H*$_3$	3.68	—	—	3.63
2,18-CH$_3$	1.85 1.86	1.87 1.89	1.85	1.54
3,17-C*H*$_2$CH$_3$	2.48[a]	2.48[a]	2.48[a]	2.37[a]
3,17-CH$_2$C*H*$_3$	1.12[b] 1.13[b]	1.13[b]	1.11[b]	1.04[b]
5,15-CH=	5.95 6.05	5.98 6.09	6.04	5.91
7,13-CH$_3$	2.22 2.23	2.09 2.22	2.24	2.18
β,β′-CH	3.43[c]	3.46[c] 2.47[j]	3.45[o]	3.53[s]
β,β′-CH$_3$	1.32[d] 1.35[d]	1.32[d]	1.35[p]	1.35[t]
α,α′-CH$_2$	2.69,[e] 3.12[f] 2.73,[g] 3.00[h]	2.69,[k] 3.11[l] 1.51,[m] 0.93[n]	2.70[q] 3.08[r]	2.74[u] 2.80[v]
10-CH$_2$	3.95[i] 4.06[i]	4.00[i] 4.07[i]	4.06	4.13

[a] q, J=7.6 Hz; [b] t, J=7.6 Hz; [c] ABX, m; [d] d, J=7.3 Hz; [e] ABX, ^{3}J=2.7, ^{2}J=18.2 Hz; [f] ABX, ^{3}J=12.2, ^{2}J=18.2 Hz; [g] A′B′X′, ^{3}J=4.5, ^{2}J=16.1 Hz; [h] A′B′X′, ^{3}J=12.2, ^{2}J=16.1 Hz; [i] AB, ^{2}J=15.9 Hz; [j] β′-CH$_2$, m; [k] ABX, ^{3}J=3.2, ^{2}J=17.7 Hz; [l] ABX, ^{3}J=12.3, ^{2}J=17.7 Hz; [m] α′-CH$_2$, m; [n] α′-CH$_3$, t, J=7.3 Hz; [o] ABX, ^{3}J=3.0, 12.3, 7.4 Hz; [p] d, J=7.4 Hz; [q] ABX, ^{3}J=3.0, ^{2}J=18.2 Hz; [r] ABX, ^{3}J=12.3, ^{2}J=18.2 Hz; [s] q, J=7.6 Hz; [t] d, J=7.1 Hz; [u] ABX, ^{3}J=6.7, ^{2}J=15.3 Hz; [v] ABX, ^{3}J=9.2, ^{2}J=15.3 Hz.

steric interaction.[8a,13] Assuming that intramolecular hydrogen bonding remains a potent conformation-stabilizing force in **1** and **2**, the presence of β(*S*) methyl groups might thus be expected to force a resolution of these pigments too.

Experimental evidence for the foregoing conclusions comes from ^{1}H{^{1}H} steady-state difference NOE and pulsed field gradients (PFG) transient NOE[14] experiments on **1** and **2**. In both mesobilirubin-XIIIα analogs, strong ^{1}H{^{1}H}-NOE enhancements found between the pyrrole NH and lactam NH, as well as between C(5)/C(15)–vinyl hydrogens and C(3)/C(17)–CH$_2$CH$_3$ and C(7)/C(13)–CH$_3$ confirmed the *syn*-Z conformation of dipyrrinone moieties. The moderate NOE between β,β′-methine hydrogens (3.43 ppm) and C(10)–CH$_2$ (3.95, 4.06 ppm) and also between β,β′-methyl hydrogens (1.32, 1.35 ppm) and C(7)/C(13)–CH$_3$ (2.22, 2.23 ppm) indicated a preference for the ***M*** helical conformation of **1**. The intramolecular hydrogen bonding in **1** is directly proven by an NOE relating the acid COO*H* and more deshielded lactam N*H* (10.85 ppm) protons (1.5%). This, however, is much weaker than that found for **3** (6.2%) possessing a full complement of hydrogen bonds. Similar irradiation of the acid COO*H* proton of **2** did not produce measurable enhancement of the lactam

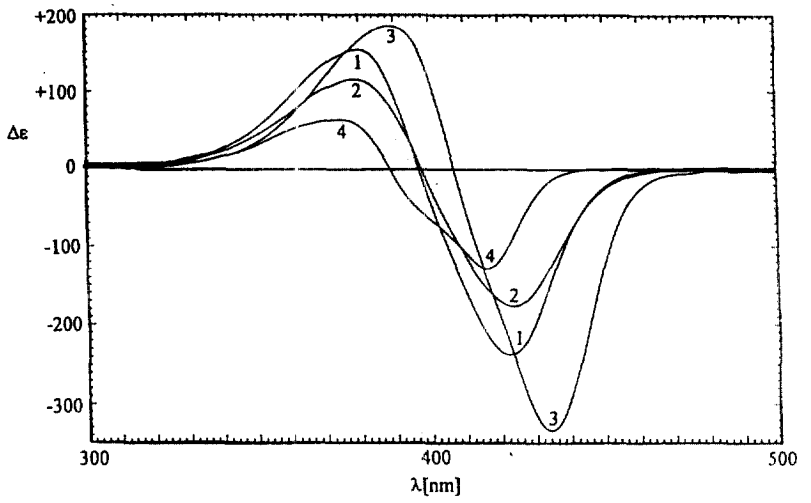


Figure 2. Circular dichroism of 1.5×10^{-5} M solutions of bilirubin analogs in chloroform: **1**, spectrum 1; **2**, spectrum 2; and 3, spectrum 3; **4**, spectrum 4.

N*H* signal and vice versa. The preferred ***M***-helical conformation of **2** was confirmed by a weak NOE between β-CH_3 (1.32 ppm) and C(7)–CH_3 (2.22 ppm). The last is weaker than the corresponding enhancement in **1**, also much weaker than that in **3**, and better observed using the PFG experiment.

Stereochemistry and circular dichroism

With the introduction of a stereogenic center on the propionic side chains of **1** and **2**, one may expect, at a minimum, modest optical activity from a π–π* excitation in the dipyrrinone perturbed by dissymmetric vicinal action.[15] However, if intramolecular hydrogen bonding prevails in **1** and **2**, as predicted by NMR and HPLC, the conformational enantiomerism shown in Figure 1B may be driven toward the ***M***-helical conformer by equilibrium-displacing perturbations of an intramolecular origin: the β(*S*) methyl substituents. If the predicted forced displacement of the ***M*** ⇆ ***P*** equilibrium is achieved, then the two dipyrrinone chromophores will be held in a fixed chiral geometry (***M*** or ***P***), depending on the *R,S* stereochemistry at β and β′; and a strong exciton chirality[2,16] interaction between them should lead to strong optical activity. Detection of optical activity (and an excess of the ***M*** or ***P*** chirality conformer) has been accomplished by circular dichroism (CD) spectroscopy on the parent acid enantiomers (**3** and *ent*-**3**), which are found to exhibit very large bisignate Cotton effects with Δε values in the range 200–400 L M^{-1} cm^{-1} for the pure enantiomers.[8a] Intense bisignate CD Cotton effects were also seen (Figure 2) for **1** and **2**—in complete agreement with the predictions of the allosteric model and exciton coupling theory.[16,17] As shown for a wide range of solvents (Table 3), β(S) enantiomers, **1**–**4** all exhibit intense bisignate CD spectra that are characteristic of the ***M***-helicity conformation.

According to exciton chirality theory,[6] the signed order of the bisignate CD Cotton effects may be used to predict the relative orientation of the two electric dipole transition moments, one from each dipyrrinone of the rubin. Thus, a positive exciton chirality [long wavelength (+) Cotton effect followed by a (−) short wavelength Cotton effect] corresponds to a positive torsion angle between the transition dipoles, and a negative exciton chirality [long wavelength (−) Cotton effect followed by a (+) short wavelength Cotton effect] corresponds to a negative torsion angle. The ***M***-helicity conformer of Figure 1B is predicted to have a negative exciton chirality; the ***P***-helicity is predicted to have a positive exciton chirality.

In all solvents except $(CH_3)_2SO$, **1** and **2** exhibit a negative exciton chirality, suggesting a

Table 3. Solvent dependence of the circular dichroism spectra of pigments **1–4** (1.5×10^{-5} M solutions at 22°C)

Pigment	Solvent	ϵ^a	CD			UV	
			$\Delta\epsilon^{max}(\lambda_1)$	λ_2 at $\Delta\epsilon=0$	$\Delta\epsilon^{max}(\lambda_3)$	ϵ^{max}	λ(nm)
1	Benzene	2.3	+152 (380)	395	-254 (420)	52,700	416
2			+111 (379)	396	-178 (423)	41,900	415
3			+191 (390)	406	-362 (434)	60,000	432
4			+ 87 (372)	385	-199 (412)	56,300	388
1	$CHCl_3$	4.7	+153 (380)	397	-238 (422)	53,300	418
2			+115 (379)	398	-178 (424)	43,800	416
3			+186 (389)	407	-337 (434)	55,800	431
4			+ 63 (376)	389	-131 (416)	59,300	416
1	THF	7.3	+133 (378)	395	-210 (419)	53,000	414
2			+127 (379)	396	-179 (420)	44,400	412
3			+188 (390)	406	-338 (433)	57,900	431
4			+ 24 (371)	384	- 56 (412)	52,500	387
1	CH_2Cl_2	8.9	+153 (378)	395	-235 (420)	52,300	415
2			+105 (377)	395	-165 (422)	42,300	415
3			+180 (392)	407	-319 (433)	56,400	430
4			+ 52 (370)	386	-105 (413)	54,400	391
1	$CH_3(CH_2)_2OH$	20.1	+108 (383)	398	-183 (424)	61,300	425
2			+ 47 (386)	404	- 60 (429)	53,200	429
3			+169 (388)	406	-253 (431)	57,600	426
4			+ 20 (374)	393	- 46 (420)	57,400	420
1	$(CH_3)_2CO$	20.7	+134 (376)	394	-209 (418)	50,300	412
2			+120 (377)	396	-168 (420)	42,800	411
3			+182 (387)	404	-322 (430)	57,100	427
4			+ 21 (367)	385	- 35 (412)	56,400	380
1	CH_3CH_2OH	24.3	+104 (382)	400	-161 (425)	55,100	422
2			+ 60 (386)	403	- 77 (429)	50,700	426
3			+168 (389)	405	-284 (434)	57,600	426
4			+ 15 (376)	393	- 30 (421)	56,800	421
1	CH_3OH	32.6	+ 98 (380)	398	-153 (424)	55,800	422
2			+ 56 (382)	402	- 74 (427)	51,000	425
3			+177 (386)	405	-285 (431)	60,800	425
4			+ 16 (371)	390	- 43 (419)	57,800	421
1	CH_3CN	36.2	+134 (375)	392	-210 (417)	50,000	410
2			+114 (375)	394	-163 (419)	42,000	404
3			+181 (384)	403	-315 (429)	56,700	423
4			+ 14 (364)	385	- 21 (412)	62,800	375
1	$(CH_3)_2NCHO$	36.7	+ 43 (379)	395	- 70 (421)	53,400	420
2			+ 57 (382)	401	- 73 (424)	47,300	395
3			+165 (386)	404	-246 (429)	54,000	421
4			- 10 (379)	391	+ 12 (417)	50,100	393
1	$(CH_3)_2SO$	46.5	- 11 (378)	393	+ 18 (427)	56,000	426
2			+ 8 (384)	413	- 2 (432)	55,500	429
3			- 6 (369)	385	+ 23 (425)	56,700	425
4			- 19 (381)	398	+ 16 (425)	59,800	427
1	CH_3NHCHO	182.4	+ 85 (379)	395	-152 (422)	55,600	421
2			+ 64 (381)	398	-103 (425)	50,200	425
3			+200 (383)	400	-359 (427)	66,000	426
4			- 17 (385)	409	+ 6 (436)	47,800	397

[a] Dielectric constant from Gordon, A.J.; Ford, R.A. *The Chemist's Companion*, Wiley, NY (1972), pp 4-8.

predominance of the ***M***-helicity conformer and confirming the predictions drawn above and based on non-bonded intramolecular steric interactions. The CD intensities are 70–90% of those seen in the diacid (**3**), indicating the $\boldsymbol{M} \leftrightharpoons \boldsymbol{P}$ conformational equilibrium is displaced strongly toward ***M*** by the presence of only one intramolecularly hydrogen-bonded propionic acid group. In $(CH_3)_2SO$ solvent, where the Cotton effect intensity drops to less than 10% of the maximum values, in both **1** and **2**, as well as **3**, the favored folded conformation has become somewhat more open to accommodate attachment

of the solvent molecules.[7] As shown earlier,[2] flattening the ridge-tile leads to a reorientation of the dipyrrinone electric transition dipole moments to near parallelity (and hence to very weak bisignate Cotton effects) and a change in torsion from (−) to (+) without a change in conformational chirality. In aqueous base the Cotton effects remain strong, consistent with an *M*-helicity dianion.

Concluding comments

Intramolecular hydrogen bonding, which is characteristic of natural bilirubin and its analogs, is known to be a dominant force in determining their conformation. The current study shows that only a single propionic acid group at C(8) or C(12) is required for stabilization of the ridge-tile conformation and that when substituted with methyl groups at the β position to create a β(*S*) stereogenic center, **1** and **2** are forced by non-bonded steric interactions to adopt an *M*-helicity ridge-tile conformation.

Experimental

General

All circular dichroism spectra were recorded on a JASCO J-600 instrument, and all UV–Vis spectra were recorded on a Perkin–Elmer Lambda 12 or Cary 219 spectrophotometer. NMR spectra were obtained on GN-300 or Varian Unity Plus spectrometers operating at 300 MHz and 500 MHz, respectively. $CDCl_3$ solvent (unless otherwise noted) was used, and chemical shifts were reported in δ ppm referenced to residual $CHCl_3$ ^1H signal at 7.26 ppm and ^{13}C signal at 77.00 ppm. J-modulated spin–echo experiments (Attached Proton Test) were used to obtain ^{13}C-NMR spectra. Optical rotations were measured on a Perkin–Elmer model 141 polarimeter. HPLC analyses were carried out on a Perkin–Elmer Series 410 high-pressure liquid chromatograph with a Perkin–Elmer LC-95 UV–Vis spectrophotometric detector (set at 420 nm) equipped with a Beckman–Altex ultrasphere IP 5 μm C-18 ODS column (25×0.46 cm) kept at 34°C. The flow rate was 1.0 mL min^{-1}, and the mobile phase was 0.1 M di-*n*-octylamine acetate buffer in 5% aqueous methanol (pH 7.7 at 22°C). Radial chromatography was carried out on Merck Silica gel PF_{254} with $CaSO_4$ preparative layer grade, using a Chromatotron (Harrison Research, Inc., Palo Alto, CA) with 1, 2 or 4 mm rotors. Melting points were determined on a Mel-Temp capillary apparatus and are uncorrected. Combustion analyses were carried out by Desert Analytics, Tucson, AZ.

Commercial reagents were used as received from Aldrich or Acros. The spectral data were obtained in spectral grade solvents (Aldrich or Fisher). HPLC grade solvents (Fisher) were dried according to standard procedures[18] and distilled prior to use.

(1S,1′S)-3,17-Diethyl-8-(2-carboxy-1-methylethyl)-12-(2-methoxycarbonyl-1-methylethyl)-2,7,13,18-tetramethyl-(21H,24H)-bilin-1,19-dione (5, βS,β′S-dimethylmesobiliverdin-XIIIα methyl ester)

A mixture of optically pure dipyrrinones **8**[8a] (495 mg, 1.5 mmol) and **9**[11] (474 mg, 1.5 mmol), *p*-chloranil (1.85 g, 7.5 mmol), formic acid (33 mL), and CH_2Cl_2 (660 mL) was heated at reflux for 24 h. The volume was reduced by distillation to one-half and reflux was continued for 6 h. The mixture was then chilled overnight at −20°C. The separated solid was filtered, the blue filtrate was washed with H_2O (3×300 mL), dried (Na_2SO_4), filtered, and the solvent was removed under vacuum. The crude mixture of three verdins (**5, 6** and **7**) was separated by radial chromatography (4–6% CH_3OH/CH_2Cl_2) collecting the bright blue band with medium polarity. After removing the solvent, 324 mg (69%) of pure verdin monomethyl ester **5** was obtained. Mp 196–198°C; $[\alpha]_{436}^{20}$ −2680 (*c* 3.9×10^{-3}, $CHCl_3$); ^1H-NMR: δ 1.25 (6H, t, J=7.6 Hz), 1.27 (3H, d, J=7.1 Hz), 1.38 (3H, d, J=7.1 Hz), 1.84 (3H, s), 1.86 (3H, s), 2.15 (3H, s), 2.21 (3H, s), 2.52 (4H, q, J=7.6 Hz), 2.57 (2H, d, J=7.2 Hz), 2.65 (2H, d, J=7.2 Hz), 3.30 (1H, m), 3.54 (1H, m), 3.56 (3H, s), 6.01 (1H, s), 6.15 (1H, s), 6.92 (1H, s), 8.36 (1H, br. s), 9.31 (1H, br. s) ppm; ^{13}C-NMR: δ 8.08, 8.20, 10.43, 10.75, 14.46, 17.82, 17.90, 20.43, 20.64, 27.83, 27.89, 40.29, 41.19, 51.58, 96.76, 97.96, 115.01, 126.07, 127.48, 128.19, 128.46, 137.34, 137.94, 139.51, 140.70, 144.11, 146.85, 147.84, 171.92, 172.42, 174.64, 176.28 ppm.

(−)-(1S,1′S)-3,17-Diethyl-8-(2-carboxy-1-methylethyl)-12-(2-methoxycarbonyl-1-methylethyl)-2,7, 13,18-tetramethyl-(10H,21H,23H,24H)-bilin-1,19-dione (1, βS,β′S-dimethylmesobilirubin-XIIIα monomethyl ester)

To an ice-bath cooled solution of verdin **5** (157 mg, 0.25 mmol) in THF (20 mL) and CH_3OH (85 mL, both dry and deoxygenated) was added sodium borohydride (950 mg, 25 mmol) portionwise over 15 min. The mixture was stirred for 45 min. at room temperature, diluted with ice/water (300 mL), and acidified with CH_3COOH. The product was extracted with $CHCl_3$ (3×100 mL), washed with H_2O (3×100 mL), dried (anhyd. Na_2SO_4), filtered, and the solvent was evaporated under vacuum. The crude product was purified by radial chromatography (1–3% CH_3OH–CH_2Cl_2 by vol.). After evaporation of the corresponding pure fractions, recrystallization from $CHCl_3$–CH_3OH afforded 105 mg (67%) of pure-by-HPLC rubin **1**. Mp 237–239°C (decomp.); $[\alpha]_D^{20}$ −4170° (c 7.0×10^{-3}, $CHCl_3$); ^{13}C- and ^{1}H-NMR in Tables 1 and 2. Anal Calcd for $C_{36}H_{46}N_4O_6\cdot H_2O$ (648.8): C, 66.64; H, 7.46; N, 8.64. Found: C, 66.54; H, 6.92; N, 8.27.

Ethyl 3-(2,4-dimethyl-5-ethoxycarbonyl-1H-pyrrol-3-yl)propanoate (17)

Compound **17** was synthesized in 49% yield from the oxime of ethyl acetoacetate and ethyl 4-acetyl-5-oxo-hexanoate as described previously.[12]

3-(2,4-Dimethyl-5-ethoxycarbonyl-1H-pyrrol-3-yl)propanoic acid (16)

To a solution of diethyl ester **17** (26.73 g, 0.1 mol) in ethanol (300 mL) was added a solution of NaOH (4.40 g, 0.11 mol) in H_2O (40 mL) and the mixture was stirred for 20 h at room temperature. The ethanol was removed under vacuum, and the residue was dissolved in 0.05 M NaOH (200 mL). The solution was extracted with $CHCl_3$ (50 mL) which was discarded, and the aqueous layer was partially evaporated under vacuum to remove traces of $CHCl_3$. The mixture was cooled in an ice bath and carefully acidified with HCl (initially with 37%; at later stage with 8%) while stirring vigorously. The product was filtered, washed with H_2O until neutral, recrystallized from EtOH–H_2O and dried under vacuum to afford 22.25 g (93%) of monoacid **16**. Mp 153–156°C (lit.[19] mp 155–156°C); ^{1}H-NMR ($(CD_3)_2SO$): δ 1.22 (3H, t, J=7.0 Hz), 2.09 (3H, s), 2.13 (3H, s), 2.23 (2H, t, J=7.1 Hz), 2.50 (2H, t, J=7.1 Hz), 4.14 (2H, q, J=7.0 Hz), 10.99 (1H, s), 12.00 (1H, br. s) ppm; ^{13}C-NMR ($(CD_3)_2SO$): δ 10.38, 10.81, 14.56, 19.29, 34.89, 58.71, 115.89, 119.47, 125.84, 130.54, 160.83, 174.04 ppm.

3-(2,4-Dimethyl-5-ethoxycarbonyl-1H-pyrrol-3-yl)propanol (15)

To a cooled (−30°C) solution of acid **16** (17.95 g, 75 mmol) in dry THF (550 mL) under N_2 was slowly added over 1 h, a 1 M solution of borane in THF (85 mL, 85 mmol). The mixture was warmed over 4 h to room temperature and stirred for 16 h. Water (100 mL) was added dropwise and THF was removed under vacuum. The crude product was dissolved in $CHCl_3$ (300 mL), and washed with 0.2 M NaOH (50 mL) and H_2O (3×200 mL). The organic layer was dried over anhyd. $MgSO_4$, filtered, and the solvent was evaporated under vacuum. Recrystallization from EtOAc–hexane afforded 16.05 g (95%) of alcohol **15**. Mp 109–110°C; ^{1}H-NMR: δ 1.34 (3H, t, J=7.0 Hz), 1.70 (2H, m), 2.21 (3H, s), 2.27 (3H, s), 2.46 (2H, t, J=7.4 Hz), 3.64 (2H, m), 4.29 (2H, q, J=7.0 Hz), 8.67 (1H, br. s) ppm; ^{13}C-NMR: δ 10.55, 11.14, 14.38, 20.03, 33.33, 59.52, 62.02, 116.59, 121.07, 126.88, 130.11, 162.05 ppm; MS:*m/z* (relative intensity) 225 [M^+] (28), 180 (86), 134 (100), 120 (5), 108 (9). Anal. Calcd for $C_{12}H_{19}NO_3$ (225.3): C, 63.97; H, 8.50; N, 6.22. Found: C, 64.19; H, 8.33; N, 6.12.

3-(2,4-Dimethyl-5-ethoxycarbonyl-1H-pyrrol-3-yl)propanol p-*toluenesulfonate (14)*

To a cooled (0°C) solution of alcohol **15** (4.50 g, 20 mmol) in dry CH_2Cl_2 (45 mL) and dry Et_3N (5.6 mL, 40 mmol) was added *p*-toluenesulfonyl chloride (5.72 g, 30 mmol) over 30 min. The mixture was stirred 3 h at 0°C and 14 h at room temperature. It was diluted with CH_2Cl_2 (100 mL), washed with 2% HCl (50 mL), water (3×50 mL), dried (anhyd. $MgSO_4$), filtered, and the solvent was removed under vacuum. Recrystallization from EtOAc–hexane afforded 6.30 (83%) of tosylate **14**. Mp 114–115°C; ^{1}H-NMR: δ 1.34 (3H, t, J=7.1 Hz), 1.75 (2H, m), 2.14 (3H, s), 2.18 (3H, s), 2.41

(2H, t, J=7.3 Hz), 2.45 (3H, s), 4.00 (2H, t, J=6.2 Hz), 4.28 (2H, q, J=7.1 Hz), 7.34 (2H, d, J=8.3 Hz), 7.79 (2H, d, J=8.3 Hz), 8.56 (1H, br. s) ppm; ^{13}C-NMR: δ 10.49, 11.32, 14.53, 19.72, 21.57, 29.64, 59.61, 69.80, 116.97, 119.79, 126.78, 127.80, 129.59, 129.77, 133.13, 144.67, 161.62 ppm. Anal. Calcd for $C_{19}H_{25}NO_5S$ (379.5): C, 60.14; H, 6.64; N, 3.69. Found: C, 59.96; H, 6.62; N, 3.61.

Ethyl 3,5-dimethyl-4-propyl-1H-pyrrole-2-carboxylate (13)

A mixture of tosylate **14** (5.69 g, 15 mmol), NaI (11.25 g, 75 mmol), Zn (9.81 g, 150 mg-atoms), glyme (150 mL), and H_2O (7.5 mL) was heated at reflux for 4 h. After cooling, it was filtered through Celite, which was washed with CH_2Cl_2 (4×20 mL). The filtrate was further diluted with CH_2Cl_2 (100 mL), washed with 0.2 M sodium thiosulfate (100 mL), 3% HCl (50 mL), and H_2O (3×200 mL). After drying (anhyd. $MgSO_4$), filtration and evaporation of the solvent under vacuum, 3.04 g (97%) of pure (by GC and NMR) pyrrole **13** was obtained. An analytical sample was prepared by recrystallization from EtOAc–hexane. Mp 97–98°C; ^{1}H-NMR: δ 0.90 (3H, t, J=7.3 Hz), 1.35 (3H, t, J=7.1 Hz), 1.45 (2H, m), 2.20 (3H, s), 2.26 (3H, s), 2.33 (2H, t, J=7.5 Hz), 4.30 (2H, q, J=7.1 Hz), 8.82 (1H, br. s) ppm; ^{13}C-NMR: δ 10.57, 11.20, 13.70, 14.43, 23.83, 25.90, 59.43, 116.56, 121.84, 126.82, 130.11, 162.13 ppm; MS: *m/z* (relative intensity) 209 [$M^{+\cdot}$] (24), 180 (59), 164 (9), 134 (100). Anal. Calcd for $C_{12}H_{19}NO_2$ (209.3): C, 68.86; H, 9.15; N, 6.69. Found: C, 68.65; H, 9.29; N, 6.65.

4-Ethyl-9-propyl-3,8,10-trimethyl-1,11-dihydrodipyrrin-2-one (12)

A mixture of pyrrole carboxylate **13** (2.09 g, 10 mmol), NaOH (2.00 g, 50 mmol), EtOH (30 mL), and H_2O (10 mL) was heated at reflux for 4 h. The solvents were evaporated completely under vacuum. The residue was suspended in EtOH (40 mL), cooled to −10°C and carefully acidified with conc. HNO_3. 5-Bromomethylene-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole[12c] (2.16 g, 10 mmol) was added, and the mixture was heated at reflux for 3.5 h. It was chilled overnight at −15°C, and the precipitated product was filtered and washed with cold EtOH. The crude material was dissolved in $CHCl_3$, washed with H_2O, dried (anhyd. Na_2SO_4), filtered, and $CHCl_3$ was removed under vacuum. Two recrystallizations from minimum volume of $CHCl_3$ and CH_3OH (~50 mL) afforded 1.96 g (72%) of bright yellow dipyrrinone **12**. Mp 220–222°C; ^{1}H-NMR: δ 0.93 (3H, t, J=7.3 Hz), 1.17 (3H, t, J=7.6 Hz), 1.48 (2H, m), 1.95 (3H, s), 2.13 (3H, s), 2.36 (2H, t, J=7.5 Hz), 2.40 (3H, s), 2.55 (2H, q, J=7.6 Hz), 6.15 (1H, s), 10.38 (1H, br. s), 11.36 (1H, br. s) ppm; ^{13}C-NMR: δ 8.47, 9.64, 11.65, 13.96, 15.05, 17.92, 24.02, 26.28, 101.25, 121.14, 122.03, 122.17, 124.96, 126.70, 131.65, 148.15, 173.96 ppm. Anal. Calcd for $C_{17}H_{24}N_2O$ (272.4): C, 74.96; H, 8.88; N, 10.29. Found: C, 74.26; H, 8.84; N, 10.08.

(1S)-8-(2-Carboxy-1-methylethyl)-3,17-diethyl-12-propyl-2,7,13,18-tetramethyl-(21H,24H)-bilin-1,19-dione (10)

A mixture of optically pure dipyrrinone **9**[11] (474 mg, 1.5 mmol) and dipyrrinone **12** (409 mg, 1.5 mmol), *p*-chloranil (1.85 g, 7.5 mmol), formic acid (33 mL), and CH_2Cl_2 (660 mL) was heated at reflux for 24 h. The mixture volume was reduced by distillation to one half and reflux was continued for 6 h. The mixture was then chilled overnight at −20°C. The separated solid was filtered, the blue filtrate was washed with H_2O (3×300 mL), dried (anhyd. Na_2SO_4), filtered, and the solvent was evaporated under vacuum. The crude mixture containing three verdins **6**, **10** and **11** was separated by radial chromatography (2–6% CH_3OH–CH_2Cl_2) collecting the bright blue band with medium polarity. After removing the solvent and drying under vacuum 265 mg (62%) of pure verdin **10** was obtained. Mp 195–197°C; $[\alpha]_{436}^{20}$ −1140 (*c* 3.3×10^{-3}, $CHCl_3$); ^{1}H-NMR: δ 0.90 (3H, t, J=7.1 Hz), 1.09 (3H, d, J=7.3 Hz), 1.25 (6H, t, J=7.6 Hz), 1.49 (2H, m), 1.83 (3H, s), 1.86 (3H, s), 2.13 (6H, s), 2.51 (4H, q, J=7.6 Hz), 2.53 (4H, m), 3.19 (1H, m), 6.01 (1H, s), 6.18 (1H, s), 6.72 (1H, s), 8.46 (1H, br. s), 9.79 (1H, br. s) ppm; ^{13}C-NMR: δ 8.11, 8.25, 9.35, 10.91, 13.83, 14.55, 17.87, 17.97, 20.65, 24.65, 26.41, 27.63, 39.48, 96.79, 98.41, 114.24, 126.42, 127.36, 128.22, 128.59, 137.65, 137.76, 140.95, 144.71, 146.72, 148.04, 173.56, 175.38, 176.84 ppm. Anal. Calcd for $C_{34}H_{42}N_4O_4$ (570.7): C, 71.55; H, 7.42; N, 9.82. Found: C, 71.56; H, 7.29; N, 9.71.

(−)-(1S)-8-(2-Carboxy-1-methylethyl)-3,17-diethyl-12-propyl-2,7,13,18-tetramethyl-(10H,21H,23H, 24H)-bilin-1,19-dione (2)

To a solution of verdin **10** (171 mg, 0.3 mmol) in THF (10 mL) and CH_3OH (100 mL, both dry and deoxygenated), $NaBH_4$ (1.14 g, 30 mmol) was added over 15 min. under N_2. After 30 min. stirring, the mixture was diluted with ice/water (300 mL) and acidified with CH_3COOH. The product was extracted with $CHCl_3$ (4×70 mL), washed with H_2O (3×100 mL), dried (anhyd. Na_2SO_4), filtered, and the solvent was evaporated under vacuum. The crude material was purified by preparative TLC (3% CH_3OH/CH_2Cl_2) and after isolation of the yellow band and recrystallization from $CHCl_3/CH_3OH$, 93 mg (54%) of pure-by-HPLC rubin **2** was obtained. Mp 191–194°C (decomp); $[\alpha]_D^{20}$ −3870 (*c* 7.5×10^{-3}, $CHCl_3$); ^{13}C- and ^1H-NMR in Tables 1 and 2. Anal. Calcd for $C_{34}H_{44}N_4O_4$ (572.7): C, 71.30; H, 7.74; N, 9.78. Found: C, 71.16; H, 7.93; N, 9.63.

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