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An enantiomerically pure bilirubin. Absolute configuration of ($\alpha R,\alpha' R$)-dimethylmesobilirubin-XIIIα

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Abstract—Enantiomerically pure (+)-($\alpha R,\alpha' R$)-dimethylmesobilirubin-XIIIα **1** and its ($\alpha S,\alpha' S$) enantiomer *ent*-**1** were synthesized in ten steps from simple precursors. Resolution was achieved at an early stage in the synthesis, with a racemic monopyrrole precursor *rac*-**6** being converted to its amides **8** with (1*S*)-camphor-2,10-sultam. Resolution of **8** to 99% d.e. was accomplished in three crystallizations, and the absolute configuration of the acid **6** was deduced by X-ray crystallography of the more crystalline, diastereomerically pure amide **7**. Circular dichroism spectroscopy of **1** showed intense bisignate Cotton effects: $\Delta\varepsilon_{435}^{max} = +344$, $\Delta\varepsilon_{391}^{max} = -193$ ($CHCl_3$), as expected for a molecular exciton, and consistent with a ***P***-helical intramolecularly hydrogen-bonded ridge-tile conformation. The Cotton effect magnitudes of **1** match almost exactly those found for (−)-($\beta S,\beta' S$)-dimethylmesobilirubin-XIIIα **11** and (+)-($\alpha R,\beta' R$)-dimethylmesobilirubin-XIIIα. However, the Cotton effect of the pseudo-*meso* diastereomer ($\alpha R,\beta' S$)-dimethylmesobilirubin-XIIIα **12** is not zero. Its large positive exciton couplet and ^{1}H NMR NOE analysis confirm that an α-CH_3 exerts a greater steric demand than a β-CH_3—by a factor of ∼3.

1. Introduction

Bilirubin (Fig. 1), the lipophilic yellow pigment of jaundice, belongs to the class of pigments called 'linear tetrapyrroles'[1] but its solution and biological properties[2] do not correlate well with either the linear structure (Fig. 1A) or a porphyrin-like structure (Fig. 1B), which are predicted to be rather polar. In bilirubin, and its mesobilirubin analogs, where vinyls are replaced by ethyl while retaining propionic acids at C(8) and C(12) (Fig. 1C), the most stable conformation is bent in the middle, in the shape of a ridge-tile.[3–6] In the ridge-tile conformation, which is flexible, not rigid, each propionic acid group is engaged in intramolecular hydrogen bonding to the opposing dipyrrinone lactam and pyrrole (Fig. 1D), thus affording considerable conformational stabilization and rendering the pigments surprisingly lipophilic.[7]

The ridge-tile conformations of Fig. 1D have near-C_2-symmetry. They are dissymmetric and enantiomeric. In an isotropic medium, bilirubin and its synthetic analog mesobilirubin-XIIIα[8] thus consist of a 50:50 mixture of equilibrating conformational enantiomers. Displacement of the equilibrium toward one or the other of the enantiomers has been achieved by complexation with a chiral compound, such as quinine[9] or serum albumin,[10] and observed by circular dichroism spectroscopy (CD).

It was found earlier that selective stabilization of one enantiomer can also be achieved through intramolecular non-bonded steric interactions, e.g. when stereogenic centers are created by a single methyl substitution at either the α or the β carbons of both propionic acid chains.[11–13] Such forced 'intramolecular resolution' was first achieved in α,α′-dimethylmesobilirubin-XIIIα **1**[11] and its β,β′-dimethyl analog.[12] The absolute configuration at the β,β′ stereogenic centers of the latter was established unequivocally from an X-ray crystal structure of the resolved diastereomeric salt (with brucine) of a monopyrrole β-methylpropionic acid precursor to the central rings of the mesobilirubin. The absolute configuration of the former, resolved by partitioning into chloroform from its complex with human serum albumin in pH 9.0 solution, was deduced from the NMR ^{1}H{^{1}H}-NOE spectrum of its bis-amide from (−)-α-phenethylamine.[11] As such it does not have the same clarity as that from the crystallographic study of the β,β′-dimethylrubin. In addition the e.e. of the 'resolved' rubin was not apodictic, and that led recently to difficulties in quantitative determinations of functional group steric size using circular dichroism on the bilirubin platform.[14]

In the following, we present an improved synthesis and unequivocal determination of the absolute configura-

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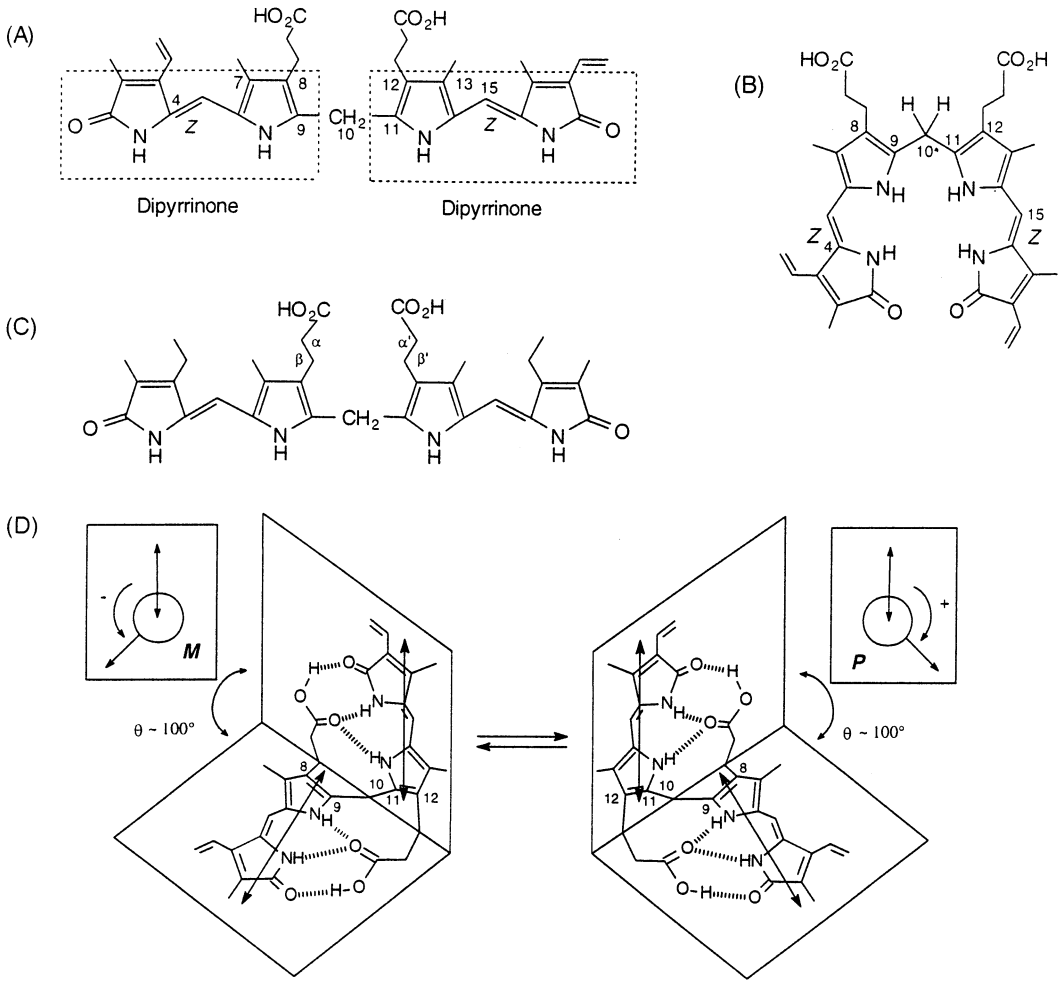


Figure 1. Bilirubin in its unstable linear (A) and porphyrin-like (B) conformations, and in its most stable ridge-tile conformations (D). (C) Mesobilirubin-XIIIα, a close analog of bilirubin that also prefers ridge-tile conformations. In (D), the enantiomeric, intramolecularly hydrogen-bonded ridge-tile conformers interconvert rapidly. The double-headed arrows represent the dipyrrinone long wavelength electric transition moment vectors (dipoles). The relative helicities (***M***, minus, or ***P***, plus) of the vectors are shown (inset) for each enantiomer. Dashed lines represent hydrogen bonds.

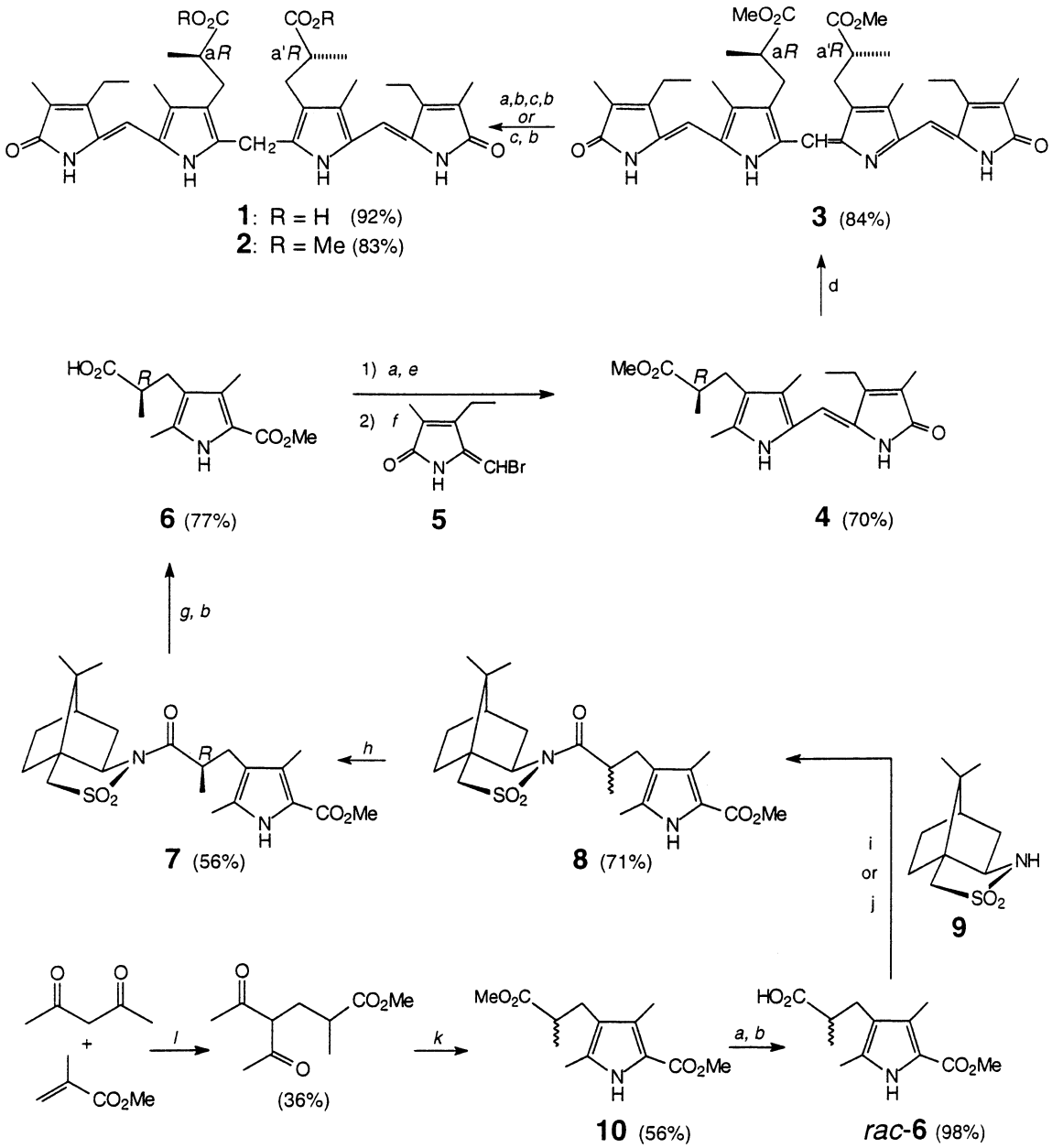
tion and e.e. of α,α′-dimethylmesobilirubin-XIIIα. In addition, we provide revised circular dichroism (CD) data for the 100% e.e. (+)-(α*R*,α′*R*) enantiomer **1** and (α*R*,β′*S*) regio isomer **12**. With CD data from the latter and previously published CD data, we are able to construct a consistent quantitative picture of alkyl (Me, Et, *i*-Pr, *t*-Bu), aryl (Bn, Ph), SMe and OMe steric size.

2. Results and discussion

2.1. Synthesis

The target rubin **1** was prepared as outlined in Scheme 1 from small components using a design strategy previously discussed.[15] The key intermediate, racemic carboxylic acid *rac*-**6**, was obtained in high yield by selective partial saponification of diester **10**, and then converted to its amides with (1*S*)-camphor-2,10-sultam **9**. Amide formation was achieved by reaction of **9** and *rac*-**6** in the presence of dicyclohexylcarbodiimide and DMAP, or by conversion of *rac*-**6** to its acid chloride (from reaction with oxalyl chloride) then coupling with the sodium salt of **9** (formed from **9** and NaH). The resulting mixture of diastereomeric amides **8** [mp 158–184°C, $[\alpha]_D^{20} = -46.6$ (*c* 1.2, $CHCl_3$)], was separated by crystallization from ethyl acetate–hexane. Three crystallizations achieved a sharp melting point (191–193°C); constant rotation: $[\alpha]_D^{20} = -92.2$ (*c* 1.2, $CHCl_3$); and ≥99% d.e. The progress of the resolution and% d.e. could be followed by ^{1}H NMR, observing the methyl region (Fig. 2) or the β-CH_2 region. The absolute configuration of the acid component of this highly resolved amide **7** was determined by X-ray crystallography (below).

Hydrolysis of sultam amides poses a problem. Harsh conditions that might lead to racemization at the propionic acid α-carbon should be avoided. Hydrolysis of the carbomethoxy group should also be avoided as the resulting pyrrole α-carboxylic acids are typically sensitive toward decarboxylation. Following Oppolzer,[16] selective cleavage of the amide group of **7** was achieved in good yield by reaction with LiOH–H_2O_2 in THF–



Scheme 1. *Reagents and conditions*: (a) NaOH; (b) HCl; (c) $NaBH_4$; (d) *p*-chloranil/HCO_2H/CH_2Cl_2; (e) HNO_3; (f) MeOH, Δ; (g) $LiOH/H_2O_2/THF/H_2O$; (h) recrystallization from EtOAc–hexane; (i) $(COCl)_2$, NaH; (j) DCC, DMAP; (k) $HON{=}C(CO_2Me)_2$, Zn, HOAc; (l) KF/EtOH, Δ.

H_2O to afford monoacid **6** and recoverable sultam **9**. Complete saponification of **6**, followed by careful acidification in cold conc. HNO_3–50% aq. $NaNO_3$ afforded the diacid of **6** in quantitative yield, and the diacid was coupled with bromomethylene–pyrrolinone **5** in refluxing CH_3OH to give dipyrrinone (–)-**4** in 70% yield. The latter was oxidatively self-coupled in the presence of *p*-chloranil to afford verdin **3** in excellent yield, and **3** was reduced to rubin dimethyl ester **2**. In a separate experiment, the verdin **3** was first saponified then reduced with $NaBH_4$ to give only (+)-**1** in overall high yield. If partial racemization had occurred during the conversion of **7** to **1**, a mixture of **1** and its (*R*,*S*)-diastereomer[11] would have been present at the final step. However, we could detect none of the latter, either chromatographically or by NMR.

2.2. Molecular geometry and absolute configuration from X-ray crystallography

Crystals of (–)-**7** were grown in ethyl acetate by diffusion of hexane vapor. Molecules of **7** pack in an orthorhombic lattice with the sultam SO_2 group lying close to one face of the pyrrole ring, and near the C(5)-CH_3. This conformation (Fig. 3) is also predicted by molecular mechanics calculations (Sybyl)[17] to be the most stable of the three staggered rotamers that follow from rotation about the amide C_α–C_β bond. In the

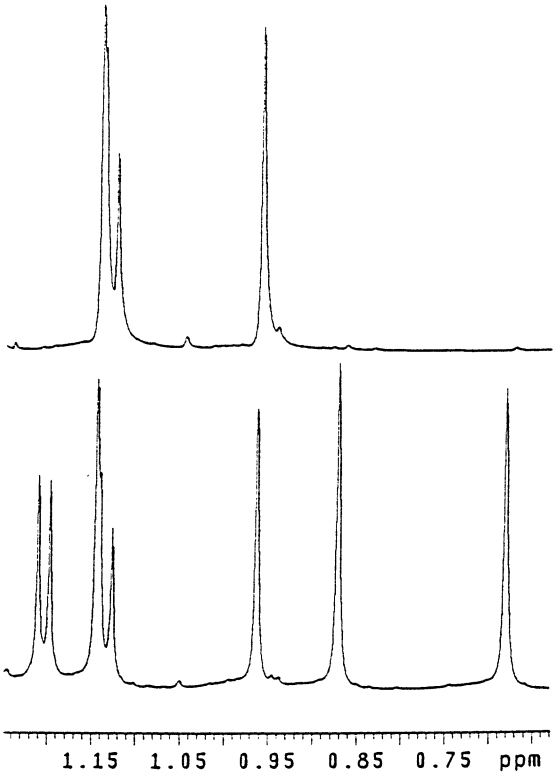


Figure 2. Partial ^{1}H NMR spectra in $CDCl_3$ of (lower) the diastereomeric camphor sultam amides **8**, showing the C(8) and C(9) camphor methyls and α-methyl region for a 50:50 diastereomer ratio, and (upper) a 99:1 diastereomer ratio in isolated **7**.

crystal, the propionic amide chain is oriented so that the pyrrole ring and amide carbonyl are *syn*-clinal rather than *anti*, and from the crystal structure one can know the relative configuration of the pyrrole and sultam components. Since the absolute configuration of the (−)-camphor-2,10-sultam is known (1*S*,2*R*,4*R*), one can readily deduce the absolute configuration of the pyrrole component of (−)-**7** as (α*R*) and thus assign the (α*R*) absolute configuration to acid **6**, with $[\alpha]_D^{20} = -51.2$ (*c* 1.2, $CHCl_3$), as shown in Scheme 1. In other ways, the molecular geometry of crystalline **7** appears unexceptional.

2.3. Local stereochemistry and pigment conformational enantiomerism

Intramolecular hydrogen bonding of the type shown in Fig. 1D is known to be the most important factor in stabilizing the ridge-tile conformation,[3–6,18,19] which is not rigid but lepidopterous.[6] Ridge-tiles with small dihedral angles (θ) are higher energy, as are ridge-tiles with larger dihedral angles.[5,6] The dynamic process of opening the dihedral angle and slipping over the barrier for the interconversion of enantiomeric ridge-tiles (Fig. 1D) is calculated to be of high energy (~20 kcal/mol)[5] and experimentally determined as rapid (3–95 s^{-1} at 50–95°C).[18] By taking advantage of intramolecular non-bonded steric interactions in the ridge-tile, one can displace the conformational enantiomeric equilibrium (Fig. 1D) toward either enantiomer simply by replacing one of the pro-stereogenic α (or β) hydrogens with a methyl group.[11–14] An (α*R*)-methyl thrusts into the C(7) or C(13) ring methyl of the dipyrrinone to which the acid is hydrogen bonded when the pigment is in the ***M***-helical conformation—but not in the ***P*** (as may be seen for the (α*R*)-CH_3 in the propionic acid at C(8) of Fig. 4). An (α*S*)-methyl would destabilize the ***P***-helical conformation but not the ***M***. Similarly, a (β*S*)-methyl would destabilize the ***P***-helical conformation but not the ***M*** (as may be seen for the (β′*S*)-CH_3 in the propionic acid at C(12) of Fig. 4). Consequently, one can tilt the equilibrium toward the ***M*** or ***P*** helicity enantiomer especially in non-polar solvents that promote hydrogen bonding, simply by the choice of local stereochemistry at the α (or β) carbon of the propionic acid chain. But one methyl group is sufficient.[20] Two methyls acting in concert are quite effective in displacing the ***M*** ⇄ ***P*** conformational equilibrium and produce very large circular dichroism (CD) Cotton effects from the pigment's

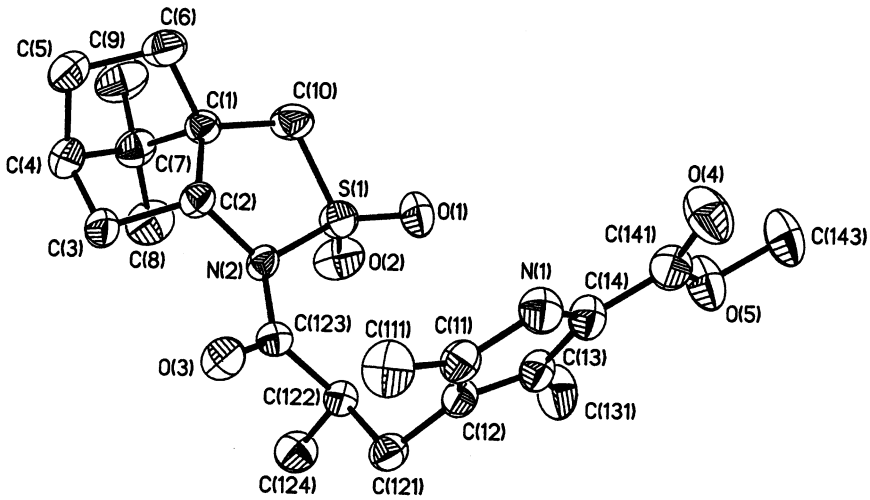


Figure 3. Crystal structure drawing and crystallographic atom numbering of amide **7**. The sultam unit lies below the pyrrole, and the stereochemistry at C(122) is evidently (*R*), given that the (−)-camphor-2,10-sultam has known (1*S*,2*R*,4*R*) absolute configuration.

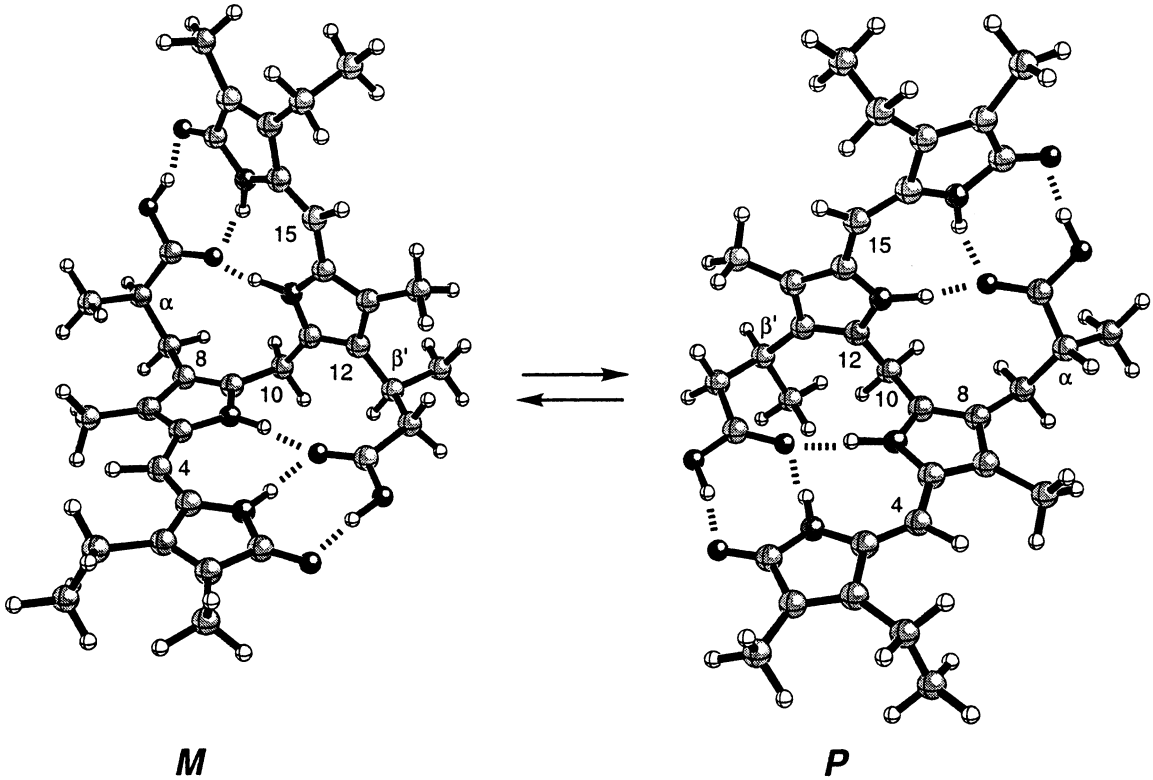


Figure 4. Ball and stick conformational representations for the ***M***- and ***P***-chirality, intramolecularly hydrogen-bonded, interconverting ridge-tile conformations of (α*R*,β′*S*)-dimethylmesobilirubin-XIIIα (**12**). One of the methyl substituents on the propionic acid side chains attached to pyrrole ring carbons C(8) and C(12) is in a sterically-crowded position, whether in the ***M*** or ***P*** conformer. When the ***M***-chirality conformer inverts into the ***P***-chirality, steric crowding of the (α*R*)-methyl by the C(7)-methyl is relieved and replaced by steric crowding of the (β′*S*)-methyl by the C(10) methylene group.

long wavelength absorption in UV–vis near 430 nm, as predicted from exciton coupling theory,[21,22] opening the door to sensitive quantitative investigations.

Previous investigations with dimethylbilirubins: (α*R*,α′*R*), (α*S*,α′*S*);[11] (β*S*,β′*S*), (β*R*,β′*R*);[12] (α*R*,β′*S*) and (α*S*,β′*S*)[13] confirmed this allosteric model and permitted a study[13] by CD spectroscopy to determine the relative importance of the steric demand of α and β methyls where they acted not in concert but in opposition, as in (α*R*,β′*S*) of Fig. 4. It was learned that an α methyl exerted a greater steric demand than a β methyl, which led to an assessment of group steric size.[14] However, certain quantitative aspects of the CD studies remained puzzling, particularly when the α,α′-methyls acted cooperatively (as in *R*,*R*, or *S*,*S*) to unbalance the conformational enantiomerism of Fig. 1D. The amplitude of the exciton CD Cotton effect was considerably lower than that when β,β′-methyls acted in unison.[13,14] We suspected that the resolved α,α′-dimethyl rubin was of <100% e.e., but we could not ascertain this with certainty or even know its e.e.[11,13]

In the current work, with precursor **6** of ≥99% e.e. and evidence against any partial racemization of the stereogenic center during the course of its conversion to **1**, we redetermined the CD Cotton effect data for **1** (Table 1). Confirming our suspicions, the new data show larger Δε values than those seen earlier.[11] Significantly, the absolute values of the Cotton effect magnitudes are essentially identical to those of (β*S*,β′*S*)-dimethylmesobilirubin-XIIIα **11**.[12] For example, the exciton CD curves of **1** and **11** are essentially mirror images (Fig. 5). Interestingly, when α and β methyls act in opposition (*anti*-chiral[14]) to control the stereochemistry of the pigment, as in the pseudo-*meso* isomer, (α*R*,β′*S*)-dimethylmesobilirubin-XIIIα **12** (Fig. 4), the (α*R*)-methyl clearly exerts a greater steric demand than the (β′*S*)-methyl and thus controls the signs of the exciton Cotton effects (Table 1). Thus, as seen in Fig. 5, positive exciton chirality CD of **12** indicates a ***P***-helicity conformation, which we estimate dominates the ***M*** by a factor of ~3 ($K_{M\rightleftarrows P}=2.97$, or 75% ***P***, and $\Delta G=-0.638$ kcal/mol at 22°C). In the other non-polar solvents (hexane, benzene, CH_2Cl_2, $ClCH_2CH_2Cl$) the equilibrium population of ***P*** is nearly the same. In the very polar, aprotic solvent *N*-methylformamide, the ***P***-helicity conformation dominates ($K_{M\rightleftarrows P}=1.64$, or 62% ***P***); and even in the polar solvent ethanol, the ***P***-helicity conformation still dominates ($K_{M\rightleftarrows P}=1.54$, or 61% ***P***). It is not entirely clear why the α-methyl should exert a greater effective steric size than a β-methyl, but the observation opened the door to assessing group steric size by CD spectroscopy.

2.4. Reassessment of functional group steric size

Previous studies indicated that one might obtain both qualitative and quantitative information on the relative steric size of functional groups (such as SCH_3 and OCH_3,[22] or Et, Bn, *i*-Pr, *t*-Bu and Ph[14]) from the CD spectra of analogs of **12**, wherein the α-substituent is drawn from those cited. The CD **A**-value, where $\mathbf{A}=\Delta\varepsilon_1^{max}(\lambda_1)-\Delta\varepsilon_2^{max}(\lambda_2)$ for $\lambda_1>\lambda_2$, of the exciton couplet, has been suggested as a useful measure of the intensity of an exciton CD couplet.[23] **A**-values from various (α*R*)-substituted, (β′*S*)-methylmesobilirubins in CH_2Cl_2 solvent are compiled in Table 2. The positive CD **A**-values observed for each example except OCH_3 confirm that the (α*R*)-substituent dominates the (β′*S*)-methyl, i.e. it is at least as large in steric size as a methyl group (as discussed in the preceding section). The negative CD **A**-value of the (α*R*)-OCH_3, (β′*S*)-CH_3 rubin indicates that the (β′*S*)-CH_3 exerts a greater steric demand than an (α*R*)-OCH_3, consistent with the α-

Table 1. CD and UV–vis spectral data from (α*R*,α′*R*), (α*R*,β′*S*) and (β*S*,β′*S*)-dimethylmesobilirubins-XIIIα at 22°C[a]

Pigment	Solvent	ε[b]	A[c]	CD			UV	
				$\Delta\varepsilon_1(\lambda_1)$	$\Delta\varepsilon_2(\lambda_2)$	λ at Δε=0	ε^{max}	λ (nm)
α*R*,α′*R*	Hexane	1.9	+615	+422 (430)	−193 (390)	403	64,700	438
							56,800	417
α*R*,β′*S*			+340	+229 (428)	−111 (386)	401	61,600	435
β*S*,β′*S*			−617	−423 (430)	+194 (388)	402	60,400	431
							53,600	411
α*R*,α′*R*	CCl_4	2.2	+586	+396 (435)	−190 (391)	408	63,100	440
α*R*,β′*S*			+319	+216 (433)	−103 (391)	406	59,900	440
β*S*,β′*S*			−572	−393 (434)	+179 (392)	406	60,800	435
α*R*,α′*R*	Benzene	2.3	+565	+370 (434)	−195 (391)	407	61,200	439
α*R*,β′*S*			+277	+179 (432)	−98 (388)	406	57,000	438
β*S*,β′*S*			−553	−362 (434)	+191 (390)	406	55,400	433
α*R*,α′*R*	$CHCl_3$	4.7	+537	+344 (435)	−193 (391)	407	59,900	437
α*R*,β′*S*			+265	+165 (430)	−100 (386)	405	55,800	430
β*S*,β′*S*			−523	−337 (434)	+186 (389)	407	55,800	431
α*R*,α′*R*	THF	7.3	+544	+351 (432)	−193 (389)	406	60,900	436
α*R*,β′*S*			+115	+70 (433)	−45 (394)	412	52,400	419
β*S*,β′*S*			−526	−338 (433)	+188 (390)	406	57,900	431
α*R*,α′*R*	CH_2Cl_2	8.9	+526	+334 (432)	−192 (389)	407	60,600	435
α*R*,β′*S*			+273	+174 (431)	−99 (388)	405	57,500	433
β*S*,β′*S*			−499	−319 (433)	+180 (392)	407	54,800	430
α*R*,α′*R*	$ClCH_2CH_2Cl$	10.4	+535	+339 (433)	−196 (390)	407	59,700	435
α*R*,β′*S*			+276	+177 (432)	−99 (389)	406	56,400	434
β*S*,β′*S*			−525	−332 (433)	+193 (389)	407	57,100	430
α*R*,α′*R*	$(CH_3)_2CO$	20.7	+515	+328 (430)	−187 (387)	404	59,500	431
α*R*,β′*S*			+172	+107 (426)	−65 (384)	402	55,400	423
β*S*,β′*S*			−504	−322 (430)	+182 (387)	404	57,100	427
α*R*,α′*R*	CH_3CH_2OH	24.3	+427	+257 (431)	−170 (388)	406	59,600	431
α*R*,β′*S*			+94	+57 (424)	−37 (382)	400	55,600	425
β*S*,β′*S*			−452	−284 (434)	+168 (389)	405	57,600	426
α*R*,α′*R*	CH_3OH	32.6	+392	+238 (429)	−154 (384)	404	60,400	429
α*R*,β′*S*			+39	+23 (422)	−16 (380)	398	56,800	424
β*S*,β′*S*			−462	−285 (431)	+177 (386)	405	60,800	425
α*R*,α′*R*	CH_3CN	36.2	+490	+310 (428)	−180 (385)	403	58,400	429
α*R*,β′*S*			+157	+99 (428)	−58 (384)	403	54,500	422
β*S*,β′*S*			−496	−315 (429)	+181 (384)	403	56,700	423
α*R*,α′*R*	CF_3CH_2OH		+492	+300 (432)	−192 (385)	406	59,200	430
α*R*,β′*S*			+133	+79 (428)	−54 (382)	402	54,600	426
β*S*,β′*S*			−479	−292 (432)	+187 (386)	406	55,200	427
α*R*,α′*R*	$(CH_3)_2SO$	46.5	−52	−23 (429)	+29 (382)	403	61,900	430
α*R*,β′*S*			−57	−26 (425)	+31 (382)	401	60,400	432
β*S*,β′*S*			+29	+23 (425)	−6 (369)	385	56,700	425
α*R*,α′*R*	CH_3NHCHO	182.4	+625	+382 (427)	−243 (384)	401	66,500	430
α*R*,β′*S*			+131	+80 (427)	−51 (385)	400	62,000	430
β*S*,β′*S*			−559	−359 (427)	+200 (383)	400	66,000	426
α*R*,α′*R*	CH_3CO_2H		+458	+286 (434)	−172 (390)	408	59,000	434
α*R*,β′*S*			−136	−81 (427)	+55 (386)	403	54,200	430
β*S*,β′*S*			−469	−287 (433)	+182 (388)	407	57,700	432

[a] Conc. $\sim 1.5\times10^{-5}$ M.

[b] Dielectric constant taken from: Gordon, A. J.; Ford, R. A. *The Chemist's Companion*; Wiley: New York, 1972; pp. 4–8.

[c] $\mathbf{A}=\Delta\varepsilon_1^{max}(\lambda_1)-\Delta\varepsilon_2^{max}(\lambda_2)$.

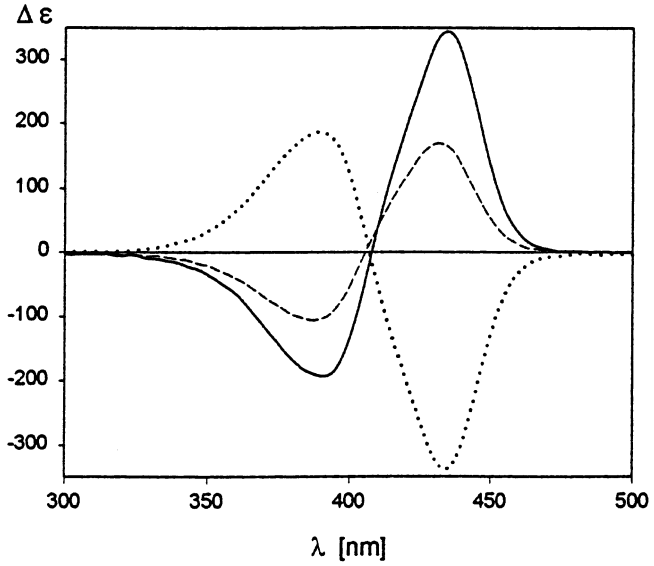


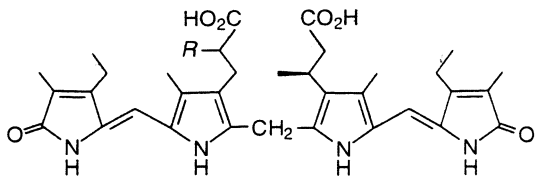
Figure 5. Circular dichroism curves of 1.5×10^{-5} M solutions of dimethylmesobilirubins-XIIIα: **1** (α*R*,α′*R*) (solid line), **12** (α*R*,β′*S*) (dashed line) and **11** (β*S*,β′*S*) (dotted line) at 22°C in chloroform.

methoxy group having a smaller steric size than an α-methyl.

Attempts to interpret the CD **A**-values in terms of Δ*G* for the ***M*⇄*P*** equilibrium (Fig. 1D) were not satisfactory; so, we simply used the CD **A**-values in order to show the relative order of steric size (Table 2), which can be compared with the order obtained from the conformational *A*-values derived from cyclohexane systems and their axial ⇄ equatorial conformational equilibria.[24] In this comparison, we note that the same relative order is followed more or less, with the notable exception of the SCH_3 group, which is found to have a larger steric size than OCH_3 (or even CH_3) by CD. We note too that isopropyl is found to have a larger steric size than phenyl by CD. It is not entirely clear why the relative steric sizes from the two different templates (bilirubin and cyclohexane) fail to agree consistently on the steric size of the group. However, it should be recognized that the gauche butane type interactions between the axial substituent on a cyclohexane ring and cyclohexane ring carbons 3 and 5 are oriented in a rather different buttressing arrangement than is the α-substituent of the bilirubin template. In the latter, the (α*R*) substituent is compressed into a C(7) or C(13) methyl group, in a face-to-face orientation, which is different from the gauche butane steric interaction encountered between an axial substituent and the C(3) and C(5) ring methylenes of chair cyclohexane. Such distinctions would doubtless come into play in a major way when the substituent has long bonds (as in SCH_3) and jammed into the C(7) or C(13) CH_3 of the bilirubin template.

Our failure to extract consistent quantitative values for steric size, such as Δ*G* for the ***M*⇄*P*** equilibrium of Fig. 4 is probably related to deformations of the ridge-tile motif. That is, although the steric size of substituents directs the equilibrium, large groups probably

Table 2. Functional group steric size from exciton chirality CD **A**-values, as compared with conformational *A*-values



Functional group (*R*)	CD **A**-values in CH_2Cl_2		Conformational *A*-values[c]	Relative steric size[d] from	
	(αβ′-*anti*-chiral)[a]	(αβ′-*syn*-chiral)[b]		*A*-values	CD **A**-values
CH_3	+273 (*R*,*S*)	+490[e] (*R*,*R*)	1.74	1.00	1.00
$CH_2C_6H_5$	+351 (*R*,*S*)	−447 (*S*,*S*)	1.76	1.01	1.29
CH_2CH_3	+374 (*R*,*S*)	−489 (*S*,*S*)	1.79	1.03	1.37
C_6H_5	+362 (*S*,*S*)	−526 (*R*,*S*)	2.87	1.65	1.33
$CH(CH_3)_2$	+453 (*S*,*S*)	−498 (*R*,*S*)	2.21	1.27	1.66
$C(CH_3)_3$	+441 (*S*,*S*)	−546 (*R*,*S*)	4.9	2.8	1.62
SCH_3	+424 (*R*,*S*)	−494 (*S*,*S*)	1.00	0.57	1.55
OCH_3	−350 (*R*,*S*)	−463 (*S*,*S*)	0.75	0.43	1.28

[a] From Table 1 and Ref. 22. The CD data for α*R*-SCH_3: $\Delta\varepsilon_{431}^{max}=+274$, $\Delta\varepsilon_{405}=0$, $\Delta\varepsilon_{386}^{max}=-150$; for α*S*-$SCH_3$: $\Delta\varepsilon_{432}^{max}=-313$, $\Delta\varepsilon_{406}=0$, $\Delta\varepsilon_{387}^{max}=+181$; for α*R*-$OCH_3$: $\Delta\varepsilon_{432}^{max}=-221$, $\Delta\varepsilon_{406}=0$, $\Delta\varepsilon_{387}^{max}=+129$; for α*S*-$OCH_3$: $\Delta\varepsilon_{431}^{max}=-290$, $\Delta\varepsilon_{405}=0$, $\Delta\varepsilon_{387}^{max}=+173$.

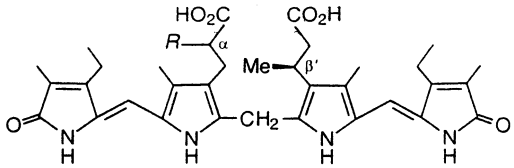
[b] From Ref. 14.

[c] From Ref. 21 (Table 2.2) by NMR: CH_3, CH_2CH_3, $CH(CH_3)_2$ ($CFCl_3$–$CDCl_3$, 300 K), $CH_2C_6H_5$ (CD_2Cl_2, 202 K), $C(CH_3)_3$ (CD_2Cl_2, 153 K), C_6H_5 (CD_2Cl_2, 173 K), OCH_3 ($CFCl_3$, 180 K), SCH_3 [$(CD_3)_2CO$/CHCl=CCl_2, ~180 K].

[d] Based on CD **A**-values, uncorrected for minor percents of ***M***-helical isomer in the α*R*,β′*S* isomers, where the α*R* substituent is CH_3 (22%), CH_2CH_3 (9%), C_6H_5 (8%) and $C_6H_5CH_2$ (6%)—as detected by ^1H NMR in $CDCl_3$. In this solvent, no ***M***-helical isomers could be detected when the substituents were $CH(CH_3)_2$ and $C(CH_3)_3$.

[e] New value; in Ref. 14 value is −445 for the (*S*,*S*) antipode.

Table 3. Influence of diethyl amine on the CD spectra and CD **A**-values of (α-substituted, β′*S*-methyl)-mesobilirubins-XIIIα



Configuration at α center		**A**	CD			UV	
			$\Delta\varepsilon_1(\lambda_1)$	$\Delta\varepsilon_2(\lambda_2)$	λ at $\Delta\varepsilon=0$	ε^{max}	λ (nm)
Me	*R*[a]	+277	−110 (390)	407	+167 (435)	62,000	442
						59,100[sh]	419–427
	R[b,c]	−986	+377 (394)	409	−609 (438)	62,700	444
						62,800	410
Bn	*R*[a]	+373	−145 (390)	408	+228 (435)	60,900	435
						57,700[sh]	409–423
	S[b]	+896	−348 (394)	410	+548 (439)	54,900	442
						55,200	407
Et	*R*[a]	+211	−86 (388)	407	+125 (435)	60,200	439
						57,400	411–424
	S[b]	+955	−369 (394)	409	+586 (438)	59,100	438
						59,200	403
Ph	*S*[a]	+552	−209 (392)	408	+343 (436)	66,200	439
						60,700	414
	R[b]	+1006	−386 (393)	408	+620 (437)	61,200	440
						61,200	406
i-Pr	*S*[a]	+121	−48 (388)	402	+73 (428)	67,800	437
						64,800[sh]	408–422
	R[b]	+959	−368 (393)	409	+591 (439)	58,400	442
						59,400	407
t-Bu	*S*[a]	+164	−65 (387)	402	+99 (428)	60,200	435
						58,300[sh]	410–424
	R[b]	+1053	−407 (394)	409	+646 (438)	61,400	442
						62,500	407
SMe	*R*[a]	+591	−231 (392)	409	+360 (437)	63,200	438
						60,200[sh]	413–424
	S[b]	+972	−374 (392)	407	+598 (436)	60,600	439
						60,700	405
OMe	*R*[a]	+926	−358 (396)	412	+568 (441)	57,700	443
						59,900	410
	S[b]	+916	−352 (393)	408	+564 (437)	60,200	440
						60,000	406

[a] *anti*-chiral configuration.
[b] *syn*-chiral configuration.
[c] (β′*R*)-configuration.

also distort the ridge-tile somewhat. And alterations of the ridge-tile template geometry change the relative orientation of the dipyrrinone components (and hence the relative orientation of their relevant electric dipole transition moments), leading to corresponding changes in CD magnitudes. This phenomenon has been discussed earlier.[5,25]

2.5. Chirality inversion

For the series of pigments where the α-substituent and (β′*S*)-methyl act in opposition sterically to direct the ***M*⇄*P*** equilibrium (Fig. 4), the intense exciton chirality CD Cotton effects observed for the various functional groups of Table 2 remain strong and positive with a change of solvent from CH_2Cl_2 to Et_2NH (Table 3). In some cases, the CD **A**-value is greatly increased, doubling or even tripling. Curiously, and for reasons unclear, the negative CD **A**-value seen in CH_2Cl_2 for the methoxy group becomes positive in diethylamine and greatly intensified. The data illustrate the sensitivity of the equilibrium to conformational changes. In the case of diethylamine solvent, presumably a change in the ridge-tile interplanar angle is needed in order to accommodate the formation of $Et_2NH_2^+$ salts of the propionic acids as well as to incorporate the $Et_2NH_2^+$ cation into the hydrogen-bonding matrix—as has been observed in the crystal structure of bis-isopropylammonium bilirubinate.[4]

Most unusual, but consistent with earlier reports,[25] for the examples of Table 2 where the α-functional group and (β′*S*)-methyl work in concert sterically to control the ***M*⇄*P*** equilibrium (thus leading to CD **A**-values

>445 in CH_2Cl_2 solvent), in Et_2NH solvent the exciton chirality is found to be inverted from negative to positive (Table 3). Without exception, the CD **A**-values are intensified to the range +900 to +1000. Although it is unclear why the α-*i*Pr rubin exhibits a slightly smaller **A**-value, the consistently positive sign indicates an inversion of molecular chirality, from the dominant ***M*** in CH_2Cl_2 to the dominant ***P*** in Et_2NH.[25] Just why the rubin should adopt the conformation in which both the α and β′ groups are in the (apparently) more sterically compressed sites remains unclear. Here again, one is dealing with ammonium salts, and the conformational preference may in some way be associated with the inclusion of the cation in the ridge-tile structure.

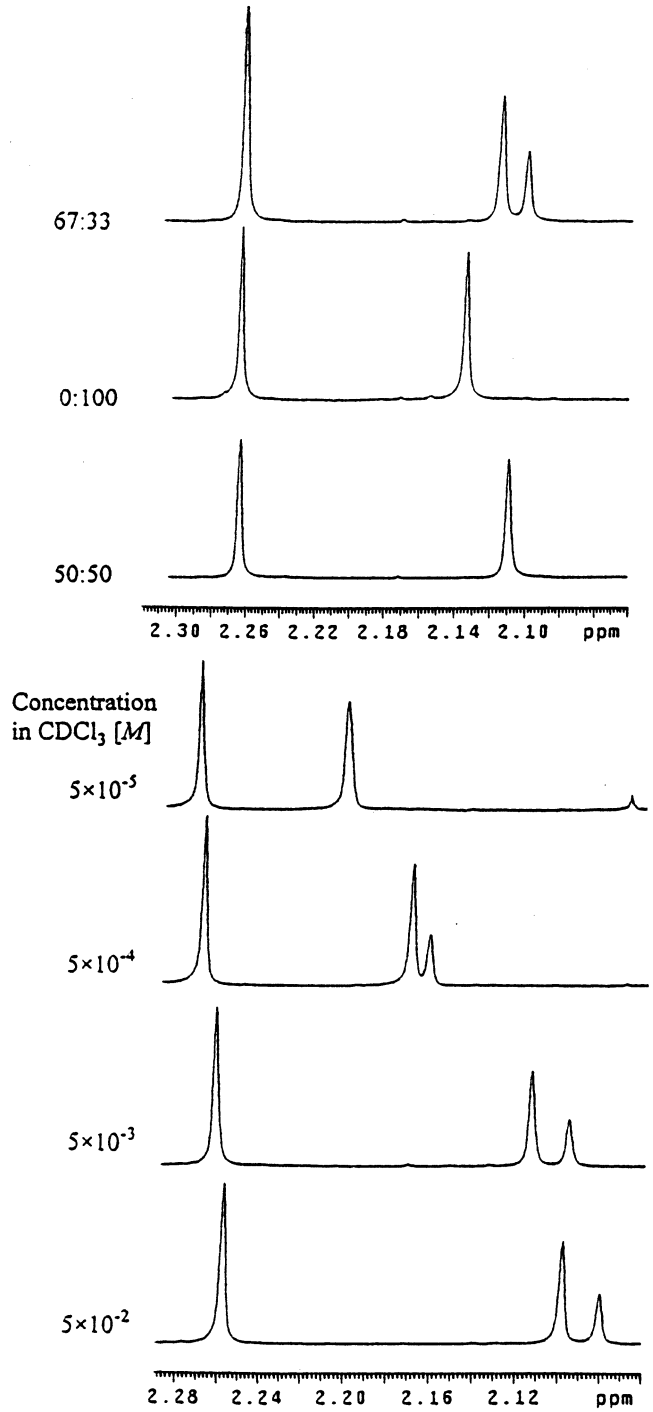


Figure 6. (A) Partial ^1H NMR spectra of the C(3) and C(5) methyl groups region in 5×10^{-3} M $CDCl_3$ solutions of pyrrole acid **6** with different enantiomeric excess. (B) Partial ^1H NMR spectra of pyrrole acid with 34% e.e. in $CDCl_3$ solutions showing the range of C(3)-CH_3 and C(5)-CH_3 signals at decreasing concentration.

2.6. Hydrogen-bonded dimers

^1H NMR studies of monopyrrole mono-acid **6** in $CDCl_3$ showed expected chemical shifts. Assignment of the C(5) methyl singlet signal as more shielded than the C(3) methyl singlet was based on NOE experiments from the NH. It was interesting to note, however, that although the C(3) methyl signal at 2.264 ppm remained invariant when comparing **6** to *rac*-**6** (Fig. 6), the C(5) methyl signal of the former was slightly more deshielded (2.135 ppm) than that of the latter (2.110 ppm) (all δ referred to strictly 5×10^{-3} M solutions). The NH signal was also slightly more deshielded in **6** (9.858 ppm) than in *rac*-**6** (9.821), but the α-methyl of **6** (1.164/1.178 ppm) was more shielded than in *rac*-**6** (1.180/1.194 ppm). These small differences in chemical shift might have escaped attention had not the 34% e.e. sample exhibited two signals for the C(5) methyl (2.102/2.117, Fig. 6A), with the C(3) methyl signal remaining at 2.264 ppm. Neither of the two C(5) signals lies at the same chemical shift as that seen in either **6** or *rac*-**6**, but the C(5) methyl of *rac*-**6** appears at the average of the two signals seen in 34% e.e. **6**.

It might be assumed that **6** forms dimers in $CDCl_3$ because in a dilution study in $CDCl_3$ (Fig. 6B), the C(5) methyl resonances of 34% e.e. **6** at 5×10^{-2} M shift upfield and coalesce at 5×10^{-5} M. Vapor pressure osmometry measurements of *rac*-**6** in $CHCl_3$ at 45°C gave an apparent molecular weight of 311 (versus that calculated for the monomer: 239), thus confirming some extent of dimer formation. In contrast, the dimethyl ester of 34% e.e. **6** shows only one signal for the C(5) methyl and no changes in chemical shift upon the 1000-fold dilution of Fig. 6B. For **6**, only one type of dimer is possible: **6**–**6** (or *R*,*R*). For *rac*-**6** there are two types: *R*,*R* (and *S*,*S*) and *R*,*S*, as shown in Fig. 7. Molecular mechanics (Sybyl) calculations indicate that the heterochiral (*R*,*S*) dimer is more stable than the homochiral (*R*,*R* or *S*,*S*) by ~2.5 kcal/mol. Thus, in *rac*-**6** one might expect a heterodimer mainly; whereas in **6**, only a homodimer is possible. The difference might account for the different chemical shifts, e.g. the C(5) methyl, seen in the ^1H NMR spectra of **6** and *rac*-**6** (Fig. 6A). In 34% e.e. **6**, one can expect to see signals for both the heterodimer (more deshielded) and homodimer (more shielded) in the ratio ~2:1, as observed.

3. Concluding comments

Methyl 4-(2′*R*-carboxypropyl)-3,5-dimethyl-1*H*-pyrrole-2-carboxylate *rac*-**6** was synthesized and resolved to 100% e.e. as its amide **7** with (1*S*)-camphor-

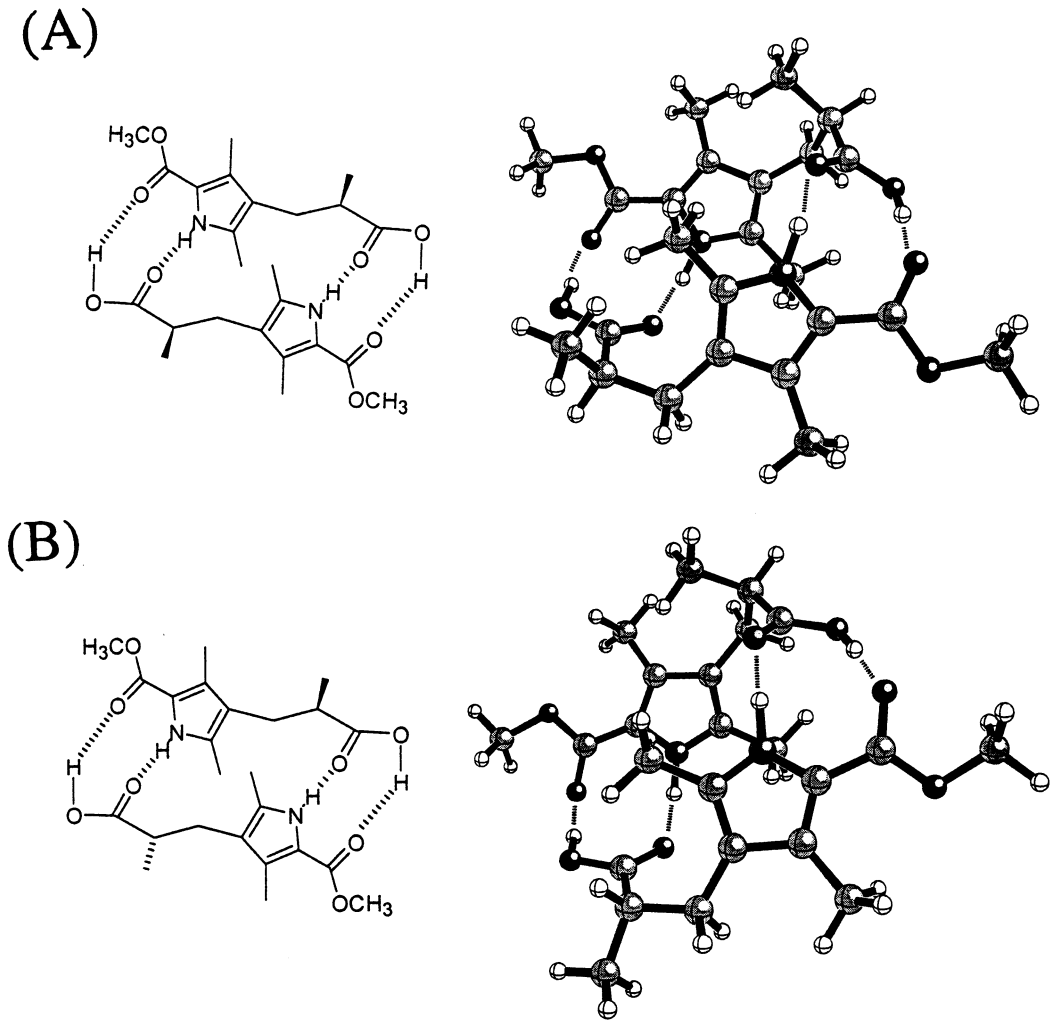


Figure 7. Line drawings (upper) and ball and stick representations of the hydrogen-bonded dimers of monopyrrole acid **6**: (A) homodimer, *R*,*R*, and (B) heterodimer *R*,*S*. The heterodimer is computed to be ~2.5 kcal/mol lower energy.

2,10-sultam. The absolute configuration of the acid component of the crystalline, resolved sultam amide was determined by X-ray crystallography, the absolute configuration of the camphor sultam being known. Both the racemic acid *rac*-**6** and resolved (αR) acid **6** form hydrogen-bonded dimers in $CDCl_3$, with the heterodimer (*R*,*S*) being favored over the homodimer. The resolved acid **6** was smoothly converted in three steps to (αR,$\alpha' R$)-dimethylmesobilirubin-XIIIα **1**, which exhibited a large positive exciton chirality CD spectrum in most non-polar solvents, but a large negative exciton chirality CD in diethylamine.

4. Experimental

All UV–vis spectra were recorded on a Perkin–Elmer Lambda-12 or Cary 219 spectrometer, and all circular dichroism (CD) spectra were recorded on a JASCO J-600 instrument. NMR spectra were obtained on Varian Unity Plus spectrometer operating at ^1H frequency of 500 MHz in $CDCl_3$ solvent (unless otherwise noted). Chemical shifts are reported in δ (ppm) referenced to the residual $CHCl_3$ ^1H signal at 7.26 ppm, and $CDCl_3$ ^{13}C signal at 77.00 ppm. A *J*-modulated spin–echo experiment (*A*ttached *P*roton *T*est), and HMBC and HMQC experiments were used to assign ^{13}C NMR spectra. Optical rotations were measured on a Perkin–Elmer model 141 polarimeter. Melting points were taken on a Mel-Temp capillary apparatus and are uncorrected. Gas chromatography–mass spectrometry analyses were carried out on a Hewlett–Packard 5890A capillary gas chromatograph (30 m DB-1 column) equipped with a Hewlett–Packard 5970 mass selective detector. Radial chromatography was carried out on Merck Silica Gel PF_{254} with gypsum preparative layer grade, using a Chromatotron (Harrison Research, Inc., Palo Alto, CA). Vapor pressure osmometry measurements were taken on a Gonotec Osmomat 070 osmometer. Combustion analyses were carried out by Desert Analytics, Tucson, AZ.

Spectral data were obtained in spectral grade solvents (Aldrich or Fisher). Commercial reagents and HPLC grade solvents were dried and purified following standard procedures.[26]

4.1. Methyl 4-acetyl-2-methyl-5-oxohexanoate

To a mixture of 214 mL (2 mol) of purified methyl methacrylate, 410 mL (4 mol) of pentane-2,4-dione and 300 mL of 95% ethanol, 58 g (1 mol) of finely ground anhydrous potassium fluoride was added during 45 min, and the contents were mechanically stirred and heated at gentle reflux for 70 h. After cooling for 1 h in an ice bath, the precipitated inorganics were separated by filtration and washed with ethyl acetate:hexane (1:1 by vol). The solvents from the filtrate were evaporated under vacuum. The residue was filtered again, and the filtrate was doubly distilled under vacuum to afford 143.6 g (36%) of β-diketone, bp 103–110°C/1.5 mmHg (lit.[11] bp 118–121°C/3 mmHg). The material was used directly in the following step.

4.2. Methyl 3,5-dimethyl-4-(2′-methoxycarbonylpropyl)-1*H*-pyrrole-2-carboxylate (10)

To a solution of 20 g (0.5 mol) of NaOH in 230 mL of glacial acetic acid cooled with ice bath, was added 80 mL (0.7 mol) of dimethyl malonate followed by a cooled solution of 103.5 g (1.5 mol) of sodium nitrite in 160 mL of water at 0°C over 3 h. After stirring for 16 h at rt, the mixture was saturated with 150 g NaCl, and the product was extracted with ether (4×200 mL). The solvent was removed under vacuum to afford a solution of dimethyl oximinomalonate in acetic acid, and this was used immediately in the pyrrole synthesis below.

In a 2 L three neck round bottom flask equipped for mechanical stirring were placed 100 g (0.5 mol) of β-diketone from above, 475 mL of glacial acetic acid, 102.5 g (1.25 mol) of anhydrous sodium acetate, and 98 g (1.5 gA) of zinc; then the mixture was warmed to 75°C. A solution of the oxime (prepared above) in 20 mL of AcOH was slowly added to the vigorously stirred reaction mixture over 2 h at such a rate as to maintain the reaction temperature at 80–85°C. The mixture was then heated at reflux for 3.5 h, after which it was poured (while hot) into 6 L of water/ice and kept overnight at 0°C. The precipitated solid was separated by filtration and washed with water (3×500 mL). The crude product was dissolved in $CHCl_3$ (800 mL) and filtered from unreacted zinc. The filtrate was washed with water (3×200 mL), dried (anhydr. $MgSO_4$), filtered, and the solvent was evaporated under vacuum. The residue was recrystallized from methanol–water (3:1 by vol) to afford 70.51 g (56%) of pure pyrrole **10**. It had mp 97–98°C; ^{1}H NMR: δ 1.11 (3H, d, $J=7.0$ Hz), 2.19 (3H, s), 2.25 (3H, s), 2.43 (1H, *A*BX, $^3J=8.3$ Hz, $^2J=14.3$ Hz), 2.57 (1H, m), 2.77 (1H, AB*X*, $^3J=6.8$ Hz, $^2J=14.3$ Hz), 3.64 (3H, s), 3.82 (3H, s), 8.60 (1H, br.s) ppm; ^{13}C NMR: δ 10.64, 11.43, 16.52, 28.11, 40.48, 50.79, 51.49, 116.73, 118.92, 127.32, 130.73, 162.22, 176.81 ppm. MS: m/z (%) 253 [$M^{+\bullet}$] (7), 222 (6), 166 (72), 134 (100). Anal. calcd for $C_{13}H_{19}NO_4$: C, 61.64; H, 7.56; N, 5.53. Found: C, 61.79; H, 7.78; N, 5.58.

4.3. Methyl 4-(2′-carboxypropyl)-3,5-dimethyl-1*H*-pyrrole-2-carboxylate *rac*-6

To a solution of 50.66 g (200 mmol) of dimethyl ester **10** in 500 mL of methanol was added a solution of 24.00 g (600 mmol) of NaOH in 100 mL of water, and the mixture was stirred for 24 h at rt. The methanol solvent was partially (~400 mL) evaporated under vacuum; then 400 mL of 0.2% aqueous NaOH was added to the residue to yield a homogeneous solution, which was cooled in an ice bath and very slowly acidified with conc. aq. HCl. The precipitated solid was collected by filtration, washed with water (3×100 mL) and dried under vacuum to afford 46.88 g (98%) of monoacid *rac*-**6**. It had mp 162–164°C; ^{1}H NMR: δ 1.18 (3H, d, $J=7.0$ Hz), 2.10 (3H, s), 2.26 (3H, s), 2.48 (1H, *A*BX, $^3J=7.4$ Hz, $^2J=14.3$ Hz), 2.61 (1H, m), 2.79 (1H, AB*X*, $^3J=7.5$ Hz, $^2J=14.3$ Hz), 3.83 (3H, s), 9.29 (1H, br.s), 11.24 (1H, very br.s) ppm; ^{13}C NMR: δ 10.82, 11.33, 16.62, 28.33, 40.80, 51.13, 116.69, 118.82, 127.72, 131.44, 162.86, 181.65 ppm.

4.4. *N*-[3-(2,4-Dimethyl-5-methoxycarbonyl-1*H*-pyrrol-3-yl)-2-methylpropanoyl]-(1′*S*)-camphor-2′,10′-sultam 8

4.4.1. Method A.[27] To a suspension of 2.39 g (10 mmol) of acid *rac*-**6** in 110 mL of anhydrous CH_2Cl_2 under N_2 was added 2.9 mL (33 mmol) of oxalyl chloride, and the mixture was heated at reflux for 3 h. The solvents were completely evaporated under vacuum, and traces of residual $(COCl)_2$ were removed by co-evaporation with dry toluene (25 mL). Simultaneously, in a separate flask equipped with side arm, (1*S*)-camphor-2,10-sultam anion was prepared in 100 mL of dry toluene by stirring **9**[28] (2.16 g, 10 mmol) with 600 mg (20 mmol) NaH (80% suspension in oil) for 2 h under N_2. This suspension was transferred through the connected side arm during 10 min to a solution of the acid chloride in 25 mL of dry toluene at 0°C, and the mixture was stirred at rt for 16 h. The reaction was quenched by adding cold water, and the product was extracted into $CHCl_3$ (4×50 mL). The extracts were washed with 1% aqueous NaOH (2×50 mL), water (3×100 mL), dried (anhydr. $MgSO_4$) and filtered. The filtrate was evaporated under vacuum, and the residue was purified by radial chromatography. Recrystallization from EtOAc–hexane afforded 3.10 g (71%) of pyrrolepropanoyl sultam **8**.

4.4.2. Method B.[29] To a solution of 21.53 g (100 mmol) (1*S*)-camphor-2,10-sultam[28] **9** in 450 mL of anhydrous CH_2Cl_2 were added 22.70 g (110 mmol) of DCC, 1.22 g (10 mmol) of DMAP and 28.72 g (120 mmol) of acid *rac*-**6**. The mixture was stirred for 16 h at rt. The separated dicyclohexylurea was removed by filtration, and washed with cold CH_2Cl_2. The filtrate was washed sequentially with 4% aqueous NaOH (2×50 mL), 3% HCl (100 mL), and water (until neutral), then dried over anhydr. $MgSO_4$. After filtration, the solvent was removed under vacuum, and the residue was purified by radial chromatography. Recrystallization from EtOAc–hexane afforded 31.34 g (72%) of **8**. It had mp 158–184°C, $[\alpha]_D^{20}=-46.6$ (*c* 1.2, $CHCl_3$); ^{1}H

NMR: δ 0.68, 0.96 (2×1.5H, 2×s), 0.87, 1.15 (2×1.5H, 2×s), 1.13, 1.20 (2×1.5H, 2×d, $J=7.0$, 6.5 Hz), 1.33 (2H, m), 1.62 (0.5H, m), 1.71 (0.5H, m), 1.88 (2.5H, m), 2.06 (1.5H, m), 2.20, 2.22 (2×1.5H, 2×s), 2.25, 2.29 (2×1.5H, 2×s), 2.48 (1H, m), 2.79 (1H, m), 3.22, 3.32 (2×0.5H, 2×m), 3.36, 3.44 (2×1H, 2×AB, $^2J=13.8$, 13.7 Hz), 3.78, 3.88 (2×0.5H, 2×dd, $^3J=4.6$, 7.6 Hz, $^3J=5.8$, 6.8 Hz), 3.802, 3.804 (2×1.5H, 2×s), 8.51, 8.56 (2×0.5H, 2×br.s) ppm; ^{13}C NMR: δ 10.58, 10.71, 11.52, 11.66, 16.92, 17.78, 19.68, 19.76, 19.79, 20.77, 26.29, 26.37, 26.38, 29.73, 32.74, 32.78, 38.16, 38.35, 40.52, 40.92, 44.59, 44.67, 47.41, 47.66, 47.92, 48.24, 50.76, 50.79, br. 53.02, 64.82, 64.97, 116.56, 116.63, 118.43, 118.68, 127.88, 128.01, int. 131.01, 162.12, 162.21, 176.03, 176.24 ppm. Anal. calcd for $C_{22}H_{32}N_2O_5S$: C, 60.52; H, 7.39; N, 6.42. Found: C, 60.74; H, 7.27; N, 6.40.

4.5. (−)-*N*-[3-(2,4-Dimethyl-5-methoxycarbonyl-1*H*-pyrrol-3-yl)-(2*R*)-methylpropanoyl]-(1′*S*)-camphor-2′,10′-sultam 7

To a warm solution of 21.83 g (50 mmol) of the 1:1 diastereomeric mixture **8** in 60 mL of ethyl acetate was slowly added 35 mL of hexane, then seed crystals of the pure (2*R*)-**7** diastereomer were added. The solution was maintained warm for ~1 h until the initial fast crystallization subsided, the mixture was slowly cooled and kept at rt for 24 h. The crystals were separated by filtration to afford 12.74 g of a fraction enriched to 75% d.e. of (2*R*)-**7**. This fraction was dissolved at reflux in 75 mL of EtOAc, and while cooling it very slowly, seeds of pure (2*R*)-**7** were added followed by hexane added in small portions (40 mL over 6 h). After 24 h, the crystalline fraction (8.56 g) was separated and subjected to another recrystallization while maintaining the same ratios of solvents. The third recrystallization gave 6.15 g (56%) of the (2*R*)-**7** diastereomer, with 99% d.e. determined by ^{1}H NMR. It had mp 191–193°C; $[\alpha]_D^{20}=-92.2$ (*c* 1.2, $CHCl_3$); ^{1}H NMR: δ 0.96 (3H, s), 1.13 (3H, d, $J=7.0$ Hz), 1.14 (3H, s), 1.36 (2H, m), 1.89 (3H, m), 2.05 (2H, m), 2.22 (3H, s), 2.28 (3H, s), 2.47 (1H, *A*BX, $^3J=9.0$ Hz, $^2J=14.3$ Hz), 2.79 (1H, A*B*X, $^3J=5.5$ Hz, $^2J=14.3$ Hz), 3.22 (1H, AB*X*Y$_3$, m), 3.41, 3.47 (1H, AB, $^2J=13.8$ Hz), 3.80 (3H, s), 3.87 (1H, dd, $^3J=6.0$, 6.5 Hz), 8.64 (1H, br.s) ppm; ^{13}C NMR: δ 10.69, 11.60, 17.74, 19.75, 20.74, 26.34, 26.35, 32.70, 38.32, 40.88, 44.56, 47.63, 48.21, 50.72, 52.98, 64.93, 116.52, 118.63, 127.95, 131.09, 162.14, 175.99 ppm.

4.6. (−)-Methyl 4-(2′*R*-carboxypropyl)-3,5-dimethyl-1*H*-pyrrole-2-carboxylate 6

To a solution of 6.55 g (15 mmol) of sultam **7** in 90 mL of purified THF cooled to 0°C was added a solution of 2.52 g (60 mmol) of lithium hydroxide monohydrate in 22 mL of H_2O. This was followed after 5 min by adding 13.5 mL (120 mmol) of 30% H_2O_2;[16,30] then the mixture was slowly warmed and stirred at rt for 16 h. The THF solvent was evaporated under vacuum, the residue was diluted with 100 mL of 1% aq. NaOH, and the camphor auxiliary was extracted with hexane (3×50 mL) and CH_2Cl_2 (2×50 mL) to give 2.30 g (71%) of recovered **9** after recrystallization from EtOH–H_2O. The material is sufficiently pure for recycling. The alkaline aqueous solution was acidified with conc. HCl to pH <3, and the pyrrole product was extracted into CH_2Cl_2–$CHCl_3$ (1:1 by vol; 4×75 mL). The combined extracts were washed with water (2×20 mL), and the solvents were evaporated to afford a residue that was purified by radial chromatography. Recrystallization afforded 2.78 g (77%) of pyrrole monoacid **6** with mp 139–141°C and $[\alpha]_D^{20}=-51.2$ (*c* 1.2, $CHCl_3$).

4.7. 5-Bromomethylene-4-ethyl-3-methyl-2-oxo-1*H*-pyrrole 5

This synthetic intermediate was prepared in four steps from ethyl acetoacetate and pentane-2,4-dione as described previously.[31] It had mp 138–140°C (lit.[31a] mp 138–139°C); ^{1}H NMR: δ 1.13 (3H, t, $J=7.7$ Hz), 1.85 (3H, s), 2.40 (2H, q, $J=7.7$ Hz), 5.90 (1H, s), 7.44 (1H, br.s) ppm; ^{13}C NMR: δ 8.27, 14.12, 17.75, 86.64, 129.45, 141.39, 145.06, 171.58 ppm. MS: *m*/*z* (%) 217, 215 [M$^{+\bullet}$] (30), 136 (100), 121 (10), 108 (85).

4.8. (−)-3-Ethyl-8-[(2′*R*)-(methoxycarbonyl)propyl]-2,7,9-trimethyl-10*H*-dipyrrin-1-one 4

A mixture of 2.39 g (10 mmol) of (2′*R*)-monoacid **6**, 2.00 g (50 mmol) of NaOH, 20 mL of ethanol, 5 mL of water, and 2.00 g of $NaNO_3$ was heated at reflux for 4 h. After cooling, the ethanol was evaporated completely under vacuum. To the residue was added 30 mL of 0.5% aq. NaOH, and the mixture was cooled to −15°C. Slow acidification with a mixture of conc. HNO_3–50% aq. $NaNO_3$ (1:5 by vol) precipitated the product, which was collected by filtration, washed with cold water (2×5 mL) and dried overnight under vacuum (P_2O_5) to afford 2.26 g (quantitative yield) of diacid. The diacid was used without further characterization in the following step.

To a solution of the diacid above in 70 mL of anhydrous methanol was added 2.16 g (10 mmol) of bromomethylene oxopyrrole **5**; then the mixture was heated under vigorous reflux for 10 h. The volume of MeOH was reduced by one-half by distillation, and the residue was chilled overnight at −15°C. The crude product was collected by filtration, washed with cold MeOH (2×10 mL) and dried under vacuum. Purification by radial chromatography and recrystallization from CH_2Cl_2–MeOH yielded 2.31 g (70%) of dipyrrinone **4**. It had mp 213–214°C (lit.[11] mp for the racemate 218–219°C), $[\alpha]_D^{20}=-55.8$ (*c* 0.3, $CHCl_3$); ^{1}H NMR: δ 1.13 (3H, d, $J=7.0$ Hz), 1.18 (3H, t, $J=7.5$ Hz), 1.95 (3H, s), 2.12 (3H, s), 2.39 (3H, s), 2.46 (1H, *A*BX, $^3J=8.7$ Hz, $^2J=14.2$ Hz), 2.55 (2H, q, $J=7.5$ Hz), 2.59 (1H, A*B*XY$_3$, m), 2.82 (1H, AB*X*, $^3J=6.3$ Hz, $^2J=14.2$ Hz), 3.67 (3H, s), 6.13 (1H, s), 10.28 (1H, br.s), 11.16 (1H, br.s) ppm; ^{13}C NMR: δ 8.48, 9.71, 11.73, 15.01, 16.56, 17.91, 28.39, 40.66, 51.55, 101.14, 118.04, 122.31, 122.41, 125.00, 126.99, 132.24, 148.30, 174.06, 177.05 ppm.

4.9. (+)-3,17-Diethyl-8,12-di[(2′*R*)-(methoxycarbonyl)-propyl]-2,7,13,18-tetramethyl-(21*H*,24*H*)-bilin-1,19-dione [(α*R*,α′*R*)-dimethylmesobiliverdin-XIIIα dimethyl ester] 3

A mixture of 661 mg (2 mmol) of dipyrrinone **4**, 1.24 g (5 mmol) of *p*-chloranil, 440 mL of CH_2Cl_2 and 22 mL of formic acid was heated at reflux for 24 h; then the volume was reduced by distillation to one-half, and reflux was continued for 6 h. The mixture was chilled overnight at −20°C. The separated solid was filtered and washed with cold CH_2Cl_2. The cold filtrate was carefully neutralized with satd aq. $NaHCO_3$, then washed with 3% aq. NaOH (2×100 mL), followed by water until neutral. It was dried (anhydr. Na_2SO_4), filtered, and the solvent was evaporated under vacuum. The residue was purified by radial chromatography and recrystallization from $CHCl_3$–hexane to afford 543 mg (84%) of verdin **3**. It had mp 217–218°C (lit.[11] mp for the racemate+*meso* diastereomers 200–205°C), $[\alpha]_{436}^{20}$ = +2190 (*c* 4.3×10^{-3}, $CHCl_3$); ^{1}H NMR: δ 1.22 (6H, t, *J*=7.6 Hz), 1.23 (6H, d, *J*=6.7 Hz), 1.83 (6H, s), 2.08 (6H, s), 2.52 (4H, q, *J*=7.6 Hz), 2.66 (4H, *AB*X, m), 3.01 (2H, AB*X*Y$_3$, m), 3.65 (6H, s), 5.93 (2H, s), 6.68 (1H, s), 8.11 (2H, br.s), 9.12 (1H, very br.s) ppm; ^{13}C NMR: δ 8.30, 9.64, 14.42, 17.09, 17.83, 28.61, 41.15, 51.75, 96.17, 114.54, 128.38, 128.45, 136.62, 139.85, 141.47, 146.69, 149.88, 172.42, 176.38 ppm.

4.10. (+)-3,17-Diethyl-8,12-di[(2′*R*)-(methoxycarbonyl)-propyl]-2,7,13,18-tetramethyl-(10*H*,21*H*,23*H*,24*H*)-bilin-1,19-dione [(α*R*,α′*R*)-dimethylmesobilirubin-XIIIα dimethyl ester] 2

To a blue solution of 129 mg (0.2 mmol) of verdin **3** in 3 mL of $CHCl_3$ and 35 mL of CH_3OH (both dry and deoxygenated with N_2) was added $NaBH_4$ (454 mg, 12 mmol) in small portions over 15 min under N_2. After 10 min, the yellow mixture was diluted with 200 mL of water. Five drops of AcOH were added followed by 5% HCl to acidify the mixture to pH ~3. The mixture was extracted with $CHCl_3$ (4×50 mL), washed with H_2O until neutral, dried (anhydr. Na_2SO_4), and filtered. The solvent was evaporated under vacuum, and the residue was purified by radial chromatography. Recrystallization (CH_2Cl_2–MeOH) afforded 107 mg (83%) of rubin dimethyl ester **2**. It had mp 244–246°C (dec.) (lit.[32] mp 230°C (dec.)); $[\alpha]_D^{20}$ = +240 (*c* 5.0×10^{-3}, $CHCl_3$); ^{1}H NMR: δ 1.00 (6H, t, *J*=7.7 Hz), 1.13 (6H, d, *J*=6.5 Hz), 1.44 (6H, s), 2.09 (6H, s), 2.32 (4H, q, *J*=7.7 Hz), 2.62 (4H, m), 2.99 (2H, m), 3.66 (6H, s), 4.11 (2H, s), 5.91 (2H, s), 10.29 (2H, br.s), 10.69 (2H, br.s) ppm; ^{13}C NMR: δ 7.71, 9.91, 14.72, 16.48, 17.76, 22.77, 28.72, 41.00, 51.52, 100.19, 117.74, 123.05, 123.44, 123.94, 128.85, 131.41, 146.99, 174.28, 177.01 ppm.

4.11. (+)-3,17-Diethyl-8,12-di[(2′*R*)-(carboxy)propyl]-2,7,13,18-tetramethyl-(10*H*,21*H*,23*H*,24*H*)-bilin-1,19-dione [(α*R*,α′*R*)-dimethylmesobilirubin-XIIIα] 1

To a solution of 161 mg (0.25 mmol) of verdin dimethyl ester **3** in 90 mL of THF–MeOH (1:1 by vol, both deoxygenated with N_2) was added 0.2 M aq. NaOH (90 mL), and the mixture was stirred at 50°C for 4 h under N_2. After cooling it was diluted with 30 mL of 0.2 M NaOH and extracted with 75 mL of $CHCl_3$ which was discarded. The alkaline green–blue solution was acidified with 10% HCl to pH ~3, and the product was extracted with CH_2Cl_2 (4×100 mL). The extracts were washed with water (2×100 mL), dried (anhydr. Na_2SO_4), and filtered. The solvent was evaporated under vacuum to give a quantitative yield of verdin diacid, which was used without delay and characterization in the following reduction step.

To a solution of the blue diacid above in 70 mL of dry and deoxygenated MeOH was added 946 mg (25 mmol) of sodium borohydride in small portions over 30 min while purging the mixture with N_2. After 15 min more stirring the yellow solution was diluted with 150 mL of H_2O. Then AcOH (2 mL) was added followed by enough 5% HCl to bring the pH ~3. The product was extracted into $CHCl_3$ (4×75 mL), washed with H_2O (3×75 mL), dried (anhydr. Na_2SO_4), and filtered. The solvent was evaporated under vacuum, and the residue was purified by radial chromatography. Recrystallization from CH_2Cl_2–MeOH afforded 142 mg (92%) of rubin diacid **1**. It had mp 313–315°C (dec.) (lit.[11] mp for the racemate 290°C (dec.)) and $[\alpha]_D^{20}$ = +5530 (*c* 5.0×10^{-3}, $CHCl_3$) (lit.[11] $[\alpha]_D^{20}$ = +4500 (*c* 5.4×10^{-4} M, CH_2Cl_2)); ^{1}H NMR: δ 1.12 (6H, t, *J*=7.5 Hz), 1.45 (6H, d, *J*=7.3 Hz), 1.86 (6H, s), 2.15 (6H, s), 2.42 (2H, AB*X*, ^3J=2.8 Hz, ^2J=14.5 Hz), 2.48 (4H, q, *J*=7.5 Hz), 2.90 (2H, *A*BX, ^3J=12.0 Hz, ^2J=14.5 Hz), 3.04 (2H, A*B*XY$_3$, m), 4.04 (2H, s), 6.05 (2H, s), 9.09 (2H, br.s), 10.54 (2H, br.s), 13.67 (2H, br.s) ppm; ^{13}C NMR: δ 7.92, 10.25, 14.86, 17.82, 19.65, 22.14, 28.03, 39.14, 100.61, 119.23, 123.20, 123.90, 124.13, 128.24, 133.14, 148.40, 174.89, 182.34 ppm.

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