Total Synthesis of the Urobiliverdin and Coprobiliverdin Isomers

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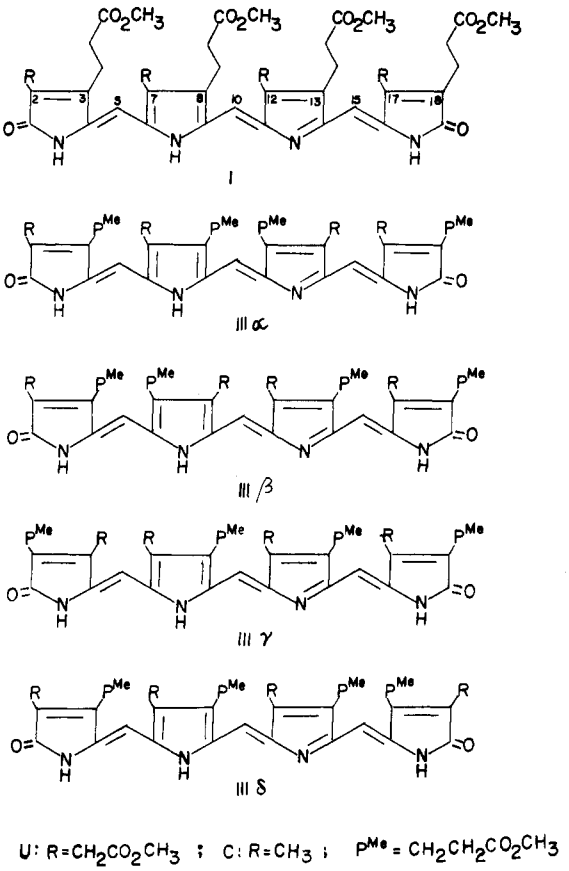
The total synthesis of the five urobiliverdin isomers which can be derived from the oxidation of uroheme I and uroheme III was achieved, as well as the synthesis of the five coprobiliverdin isomers which can be derived from the oxidation of coproheme I and coproheme III. The biliverdins were obtained by oxidation of the corresponding 1,19-bis[(*tert*-butyloxy)carbonyl]-b-bilenes. The latter were obtained by condensation of the appropriate 5'-[(*tert*-butyloxy)carbonyl]-5-formyldipyrrylmethanes with the α-unsubstituted 2-[(*tert*-butyloxy)carbonyl]dipyrrylmethanes. Urobiliverdin I was found to be identical with bactobilin, a bile pigment isolated from anaerobic microorganisms.

In a preliminary report of this work[1] we have shown that the bile pigment isolated from prokaryotes and for which the name bactobilin was proposed[2] was identical with urobiliverdin I[3] (Chart I). This was achieved by comparison of bactobilin with a synthetic sample, the synthesis of which raised the problem of an efficient procedure to prepare urobiliverdin and coprobiliverdin isomers (Chart I). All the biliverdin derivatives isolated until now from eukaryotes (biliverdins IXα and IXβ, the prosthetic groups of phycobilins and phytochrome) are derived from the biological oxidation of heme IX[4] and are therefore bilin-1,19-diones[5] substituted with propionic acid residues at C-8 and C-12 (see Chart I) and with methyl, vinyl, or ethyl residues at the other β-positions of the bilindione molecule. The isolation of bactobilin, a bilitriene possessing four acetic and four propionic acid side chains, suggested that the well-known biological oxidation of IX to biliverdin IXα which takes place in plants and animals could also take place with the earlier intermediates of the heme biosynthetic pathway, namely, with the uroporphyrin and coproporphyrin isomers. There is only one heme IX isomer in living cells (iron-protopporphyrin IX) while there are two natural isomers (I and III) of uroporphyrin and coproporphyrin;[6] therefore, the oxidative breakdown of the natural uroporphyrins and coproporphyrins could give rise to ten biliverdin isomers (Chart I).

The total synthesis of the biliverdin IX type isomers and derivatives was usually achieved by base-catalyzed condensations of 3-pyrrolin-2-ones with 5-formylpyrrole-carboxylates which afforded 1(10*H*)-dipyrrinone-9-carboxylates. The latter were transformed into 9-formyl-1(10*H*)-dipyrrinone which were condensed with 9-unsubstituted 1(10*H*)-dipyrrinones to give biliverdins bearing an unsymmetrical substitution pattern.[7] However, the preparation of the dipyrrinones needed for this synthetic approach is not so straightforward when the synthesis of a bilindione structure heavily substituted with acetic and propionic acid residues is needed. Although an earlier report[8] showed the possibility of obtaining 1-(10*H*)-dipyrrinone from dipyrrylmethane-α-carboxylic acids by conversion of the latter into α-bromo (or chloro) dipyrrylmethanes followed by an hydrolysis step, it was only recently that the conversion of b-bilenes or a,c-biladiene-α,α'-dicarboxylates to biliverdins through the sequence of bromination and hydrolysis, was put on a firm basis.[9] This opened the possibility of using the b-bilenes and its precursors usually prepared as intermediates for porphyrin synthesis,[10] also as intermediates for biliverdin

Chart I. Urobiliverdin (U) and Coprobiliverdin (C) Isomers



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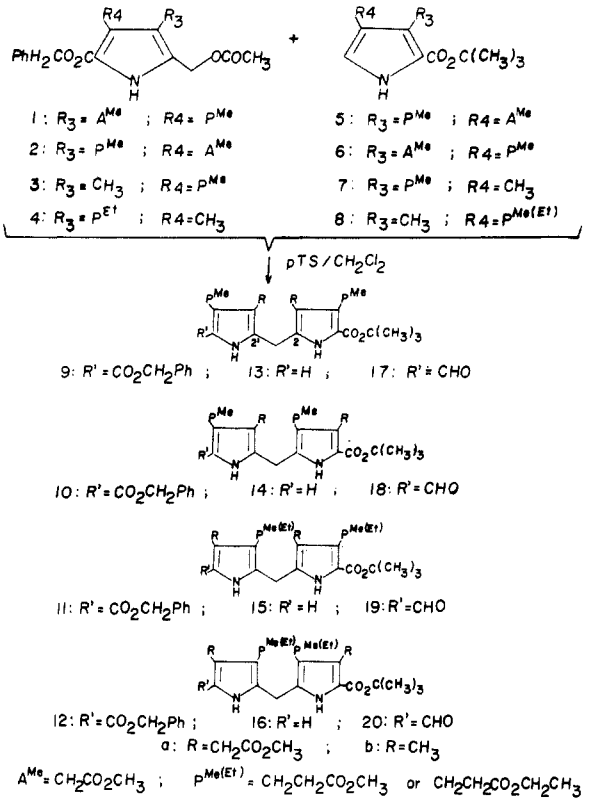
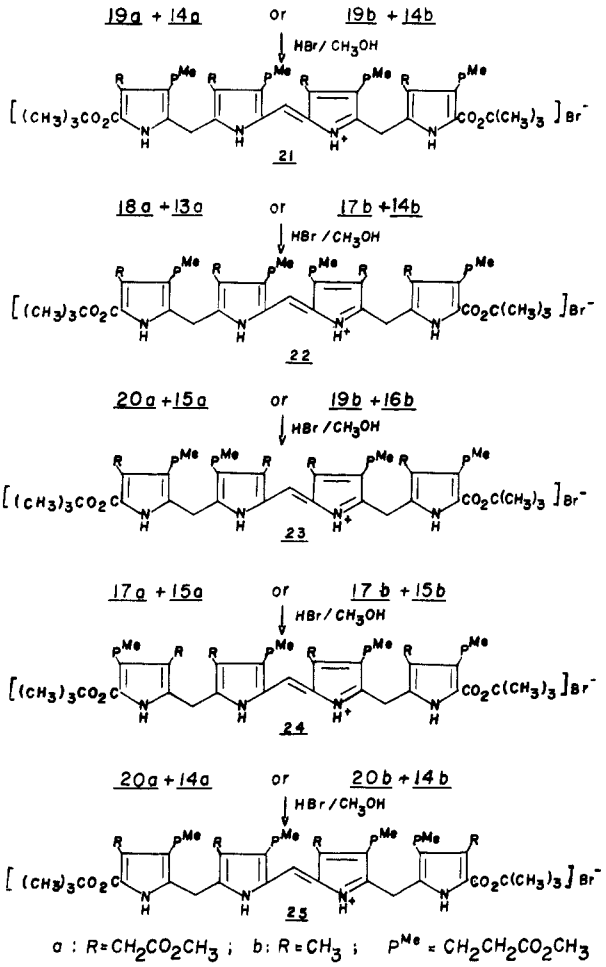
Scheme I



Scheme II



synthesis, and this report shows that indeed this approach allowed the efficient synthesis of urobiliverdin and coprobiliverdin isomers. The latter were recently obtained as mixtures of isomers by the chemical "biomimetic oxidation" of uroheme III and copropheme III,[11] but the yields of the biliverdin mixtures was very low (much lower than that obtained during the oxidation of hemin IX to the biliverdin IX isomers[12]), while the separation of the isomers is still a complex procedure.

The ten biliverdins depicted in Chart I were obtained by a synthetic approach which was based on the oxidation of the corresponding b-bilenes. Eight 5′-[(*tert*-butyloxy)carbonyl]-5-(benzyloxycarbonyl]dipyrrylmethanes **9** (**a** and **b**)–**12** (**a** and **b**) were obtained by condensation of the corresponding (2-acetoxymethyl)pyrroles **1**–**4** with the α-unsubstituted pyrroles **5**–**8** (Scheme I). The synthesis of all the eight monopyrroles was reported elsewhere.[10,13] Hydrogenolysis of the benzyl residues of the dipyrrylmethanes **9**–**12** over 10% Pd/C, followed by purification of the resulting dipyrrylmethane-5′-carboxylic acids by column chromatography on TLC silica gel and decarboxylation of the latter by heating in vacuo above 200 °C for a short period, afforded the α-unsubstituted dipyrrylmethanes **13**–**16**. Formylation of the latter with the dimethylformamide–benzoyl chloride reagent afforded the 2-formyldipyrrylmethanes **17**–**20** which were then condensed with the α-unsubstituted dipyrrylmethanes **13**–**16** in the presence of 48% HBr to give the corresponding ten b-bilene hydrobromides **21** (**a** and **b**)–**25**(**a** and **b**) (Scheme II). The latter were oxidized to the corresponding biliverdin esters (Chart I) with bromine and trifluoroacetic acid. The yields of the oxidations of the b-bilenes to the biliverdins was poor (around 10%), and better yields were obtained in the coprobiliverdin series than in the urobiliverdin series.

All the coprobiliverdin tetramethyl ester isomers as well as urobiliverdins I, IIIα, IIIβ, and IIIγ octamethyl esters were crystalline solids, while urobiliverdin IIIδ octamethyl ester was an oil. The wide range of melting points found for the coproporphyrin tetramethyl isomers (I: mp 188–190 °C; IIIα: mp 190–192 °C; IIIβ: mp 135–137 °C; IIIγ: mp 202–203 °C; IIIδ: mp 92–94 °C) suggests that these isomers have crystal packings which reflect different configurational structures. It is known that the biliverdins are chromophores of high flexibility and adopt a large number of configurational, conformational, and tautomeric forms.[14]

The uro- and coprobiliverdin isomers have a UV max around 370 nm (Table I) and a vis max around 640–650 nm when registered in chloroform at 10^{-5} M concentration. The position of the two main absortion bands (particularly the vis max) are solvent and concentration dependent. In methanol, urobiliverdins had a UV max around 370 nm and a vis max around 640–650 nm (e.g., urobiliverdin IIIβ had a UV max at 369 nm and a vis max at 648; ϵ(vis)/ϵ(UV) = 0.27) and coprobiliverdins had a UV max around 365 nm and a vis max around 640 nm (e.g., coprobiliverdin IIIβ had a UV max at 362 nm and a vis max at 645 nm; ϵ(vis)/ϵ(UV) = 0.28). At a 10^{-4} M concentration in methanol the vis max had a hypsochromic shift of about 15 nm and the UV max had a bathochromic shift of about 20 nm,

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Table I. Electron Absorption Spectra of the Urobiliverdin and Coprobiliverdin Octamethyl Ester[a] Isomers

	UV		vis			UV		vis		
isomer	max	ϵ, mM	max ($CHCl_3$)	ϵ, mM	ϵ(vis)/ϵ(UV)	max	ϵ, mM	max ($CHCl_3$–HCl)	ϵ, mM	ϵ(vis)/ϵ(UV)
urobiliverdin I	372	58.0	633	15.5	0.27	370	51.7	674	44.7	0.86
								666	46.2	0.89
urobiliverdin IIIα	371	58.5	625	16.3	0.28	370	59.0	662	59.0	1.00
Urobiliverdin IIIβ	372	52.0	654	13.7	0.26	371	54.7	718	41.7	0.76
								706	40.6	0.74
								667	35.5	0.65
urobiliverdin IIIγ	372	59.6	637	15.2	0.26	371	80.6	674	45.9	0.57
								666	46.5	0.57
urobiliverdin IIIδ	372	42.0	633	11.8	0.28	371	42.8	719	28.8	0.69
								669	36.6	0.85
								664	36.9	0.86
coprobiliverdin I	368	62.8	649	17.0	0.27	366	69.0	664	44.8	0.65
coprobiliverdin IIIα	369	67.0	644	17.0	0.25	365	74.0	659	57.0	0.77
coprobiliverdin IIIβ	366	50.7	648	13.0	0.26	365	75.0	664	38.6	0.51
coprobiliverdin IIIγ	367	59.0	642	14.8	0.25	373	85.0	662	46.0	0.51
coprobiliverdin IIIδ	370	72.0	650	21.6	0.30	366	83.7	664	49.7	0.59

[a] Concentration of urobiliverdins, 3.4×10^{-5} M; concentration of coprobiliverdins, 2.15×10^{-5} M.

Table II. Proton Chemical Shifts and Assignments of Bactobilin Octamethyl Ester and the Octamethyl Esters of Urobiliverdin Isomers[a]

bactobilin[b]	isomer 1[c]	isomer IIIα[c]	isomer IIIβ[c]	isomer IIIγ[c]	isomer IIIδ[c]
6.784 (1 H, s); H-10[d]	6.784 (1 H, s); H-10[d]	6.838 (1 H, s); H-10	6.778 (1 H, s); H-10	6.708 (1 H, s); H-10	6.852 (1 H, s); H-10
6.015 (1 H, s), 5.976 (1 H, s); H-5 and H-15	6.015 (1 H, s), 5.976 (1 H, s); H-5 and H-15	5.947 (1 H, s), 5.913 (1 H, s); H-5 and H-15	6.064 (1 H, s), 5.992 (1 H, s); H-5 and H-15	5.918 (2 H, s); H-5 and H-15	6.092 (1 H, s), 6.059 (1 H, s); H-5 and H-15
3.719, 3.712, 3.693, 3.687, 3.668, 3.659, 3.653, (24 H); CO_2CH_3	3.752, 3.744, 3.727, 3.719, 3.696, 3.691, 3.688 (24 H); CO_2CH_3	3.767, 3.729, 3.710, 3.699, 3.686, 3.683, 3.658, 3.654, (24 H); CO_2CH_3	3.752, 3.728, 3.723, 3.698, 3.693, 3.685 (24 H); CO_2CH_3	3.729, 3.719, 3.712, 3.694, 3.659, 3.651, 3.645 (24 H); CO_2CH_3	3.715, 3.706, 3.688, 3.685, 3.678, 3.674, 3.657 (24 H); CO_2CH_3
3.716 (2 H, s), 3.685 (2 H, s); H-7a and H-12a	3.662 (2 H, s); 3.643 (2 H, s); H-7a and H-12a	3.613 (2 H, s); H-7a	3.645, 3.639 (6 H, b); H-8a, H-12a, H-17a	3.605, 3.603 (4 H, b); H-7a and H-12a	3.545 (4 H, b); H-7a and H-12a
		3.389 (2 H, s); H-13a			
3.635 (2 H, s); H-17a	3.555 (2 H, s); H-17a	3.300 (2 H, s); H-17a	3.383 (2 H, s); H-2a	3.594 (2 H, s); H-3a or H-17a	3.360 (4 H, b); H-2a and H-18a
3.473 (2 H, s); H-2a	3.393 (2 H, s); H-2a	3.240 (2 H, s); H-2a		3.486 (2 H, s); H-3a or H-17a	
2.923 (2 H, t); H-13a	2.952 (2 H, t); H-13a	2.875, 2.852, 2.845 (6 H, m); H-12a, and H-8a, and H-3a	2.873, 2.863, 2.841 (6 H, m); H-13a, H-7a, and H-3a	2.891 (2 H, t), 2.793 (2 H, t); H-8a and H-13a	2.948 (2 H, t); H-8a
2.847, 2.818 (4 H, m); H-3a and H-8a	2.870 (t) and 2.845 (4 H, t); H-3a and H-8a				
2.651 (2 H, t); H-18a	2.680 (2 H, t); H-18a	2.687 (2 H, t); H-18a	2.692 (2 H, t); H-18a		2.874, 2.869, 2.858, 2.853, 2.850, 2.842, 2.843 (6 H, m); H-13a, H-17a, H-3a
2.62–2.49 (8 H, m); H-3b, H-8b, H-13b, H-18b	2.606, 2.579, 2.565, 2.545 (8 H, m); H-8b, H-13b, H-3b, H-18b	2.603, 2.579, 2.563, 2.546 (8 H, m); H-3b, H-8b, H-12b H-18b	2.590, 2.571, 2.569, 2.539 (8 H, m); H-13b, H-7b, H-3b, and H-18b	2.615, 2.540, 2.517, 2.501 (12 H, m); H-2a, H-18a, H-2b, H-8b, H-13b, and H-18b	2.684, 2.670, 2.655, 2.639 (4 H, m); H-8b and H-13b
					2.591, 2.575, 2.561, 2.547, 2.542, (4 H, m); H-36 and H-17b

[a] The chemical shifts are quoted in parts per million downfield from Me_4Si. [b] The spectrum was obtained at 500 MHz in $CDCl_3$. [c] The spectra were obtained at 470 MHz in $CDCl_3$. [d] See Chart I for the numbering system. Concentration of urobiliverdins, 5×10^{-3} M.

which could be attributed to the formation of aggregates of the extended forms.[14] Upon acidification of the chloroform solutions there was a strong bathochromic shift of the vis max both in the urobiliverdin and in the coprobiliverdin isomers and a pronounced increase in the ϵ(vis)/ϵ(UV) ratio (Table I). It has been established (in studies carried out mainly with biliverdin IXα dimethyl ester) that the ratio ϵ(vis)/ϵ(UV) is in the verdins a function of their molecular extension. An increase in this ratio indicates a change from the helically coiled form (5*Z*,10*Z*,15*Z*) where the ratio is similar to that of a porphyrin to more extended forms (e.g., 5*Z*,10*E*,15*Z*) where the ratio is similar to that of a polyene.[4d,14]

The analysis of the high field ^{1}H NMR spectra of the five urobiliverdin isomers was found to be a useful analytical tool to differentiate among them and to establish beyond doubt that bactobilin was identical with urobiliverdin I. The ^{1}H NMR spectra (Table II) showed the expected low-field methine signals (H-10, H-5, H-15) where the H-10 had the lowest field values.[15] The resonances of the methylene residues of the acetate chains were extremely useful in the characterization of the isomers. There are two clearly different types of acetate and propionate residues in urobiliverdins; the exo chains which are α to the amide carbonyl groups and the endo chains which are at the other positions of the pyrrole rings. It has been shown that in the biliverdin isomers the exo methyl residues and the exo methylenes of the propionate residues bound to the pyrrole ring are always shifted to higher fields when compared with the similar endo residues.[1,12] It is also known that the b-methylenes (bound to the methoxycarbonyl residues) are always shifted to higher fields when compared with the a-methylenes (bound to the pyrrole ring). Therefore, in urobiliverdin I octamethyl ester the endo methylenes (7a and 12a) were at the lower field values (3.662 and 3.643 ppm) while the exo methylene 2a was at higher field value (3.393 ppm), and the methylene at 17a had an intermediate value (3.555 ppm). Similar criteria were used to assign the acetate methylene shifts of the other urobiliverdin isomers (Table II). In the IIIδ isomer the endo acetate methylenes 7a and 12a had a single low field value (3.545 ppm) and the exo 2a and 18a methylenes had a single high field value (3.360 ppm). The pattern of the acetate methylene resonances was so different in the urobiliverdin I, IIIα, IIIβ, IIIγ, and IIIδ isomers that it allowed to distinguish among them (Table I).

The assignment of resonance values was more straightforward in the case of the methylenes of the propionate residues. In the case of the exo propionates, the a-methylene signals (see Chart I) shift to the higher field values and sometimes overlap with the b-methylene values. Thus in urobiliverdin I octamethyl ester the 13a, 8a, and 3a methylenes were assigned to the 2.952, 2.870, and 2.845 ppm resonances, while the 18a (exo) signal was at 2.680 ppm, and the b-methylenes were in the 2.606–2.545 ppm region (Table I). In urobiliverdin IIIγ the 2a and 18a methylenes of the exo propionate residues overlapped with the b-methylene shifts at the high field values (2.615–2.501 ppm).

The comparison of the spectrum of bactobilin octamethyl ester with those of the urobiliverdin isomers clearly showed that bactobilin is identical with urobiliverdin I (Table I). The identity of urobiliverdin I and bactobilin was also established by a HPLC analysis of their free acids (Table III). Although urobiliverdins IIIγ and IIIδ were not separated from urobiliverdin I in this system, their ^{1}H NMR spectra (Table I) ruled out these structures for the natural pigment.

Table III. HPLC Analysis of Urobiliverdins I, IIIα, IIIβ, IIIγ, and IIIδ[a]

isomer	t_R (min)	isomer	t_R (min)
bactobilin	9.50	urobiliverdin IIIβ	11.00
urobiliverdin I	9.50	urobiliverdin IIIγ	9.25
urobiliverdin IIIα	12.25	urobiliverdin IIIδ	9.50

[a] Acetonitrile (2%) in 0.05 M sodium citrate bufer (pH 7) was used as solvent. Flow rate was 0.7 mL/min at 1200 psi. The urobiliverdin peaks were detected at 372 nm.

The ^{1}H NMR spectra (2×10^{-2} M) of the tetramethyl esters of the coprobiliverdin isomers (see Experimental Section) had a similar shift pattern to that discussed above. The exo methyl residues had signals around 1.80 ppm, while the endo methyls resonated at 2.25–2.0 ppm. H-10 had the lowest field signal, while H-5 and H-15 had different shifts in all the isomers except in coprobiliverdin I. The shifts of the a- and b-methylenes in the propionate residues were as expected; in coprobiliverdin IIIγ, six methylenes (the b-methylenes plus 2a and 18a) shifted to a single high field value (2.05 ppm).

The presence of urobiliverdin I in bacteria was unexpected since all the natural biliverdins in eukaryotes are type III isomers.[4] Heme oxygenase is certainly not involved in the oxidation of a hypothetical uroheme I since extensive studies on the specificity of this enzyme have shown that neither hemins of type I nor octacarboxylic hemins are substrates of the enzyme.[16] Uroheme III was recently chemically oxidized in very low yields to a mixture of the urobiliverdin isomers,[11] but the enzymatic results suggest that a uroporphyrinogen (a hexahydroporphyrin) rather than an uroporphyrin is the precursor of bactobilin.[2] The fact that the synthetic urobiliverdins are excellent substrates of biliverdin reductase[17] adds relevance to the physiological significance of bactobilin.

Experimental Section

General Procedures. Melting points were determined on a Kofler melting point apparatus and are uncorrected. ^{1}H NMR spectra were routinely recorded in $CDCl_3$ on a Varian FT-80A spectrometer. The 500-MHz spectrum was recorded on a University of Chicago built DS-1000 spectrometer equipped with a Nicolet 1180 data acquisition system. The 470-MHz spectra were recorded at 5000 Hz on a Nicolet 470-MHz (^{1}H) spectrometer with a 32K memory and processed by a Nicolet computer. Mass spectra were obtained with a Varian CH-7 spectrometer. The silica gel used in column chromathography was TLC Kieselgel (Merck). TLC was performed on precoated silica gel F-254 plates (Merck, 0.25 mm layer thickness). The substances were spotted by spraying the plates with Ehrlich's reagent (2% *p*-(dimethylamino)benzaldehyde in 6 N HCl) or by treatment with bromine vapor which gave orange or red colors with the dipyrrylmethanes.

***tert*-Butyl 3,3′-Bis[β-(methoxycarbonyl)ethyl]-4,4′-[bis(methoxycarbonyl)methyl]-5′-[(benzyloxy)carbonyl]dipyrrylmethane-5-carboxylate (12a).** A solution of 1.12 g (3.4 mmol) of pyrrole **5**,[10a] 1.49 g (3.2 mmol) of the acetate **2**,[10a] and 120 mg of *p*-toluenesulfonic acid in 60 mL of dry methylene chloride was heated at 40 °C during 5 h while being stirred with a stream of nitrogen. The solution was poured into 100 mL of

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water, the organic layer was separated, the aqueous layer was extracted with methylene chloride (3 × 50 mL), and the pooled organic solvents were washed with a saturated sodium bicarbonate solution and then with water, dried (Na_2SO_4), and evaporated to dryness in vacuo. The dipyrrylmethane **12a** was purified by filtration through a TLC silica gel column (4 × 30 cm) using 2% methanol in benzene as eluant. The fractions containing the dipyrrylmethane **12a** (monitored by TLC) were pooled and evaporated to dryness. The oily residue (1.5 g, 62%) had the following: mass spectrum, *m/e* (relative intensity) 696 (M^+, 10), 639 (M^+ – isobutylene, 15), 595 (639 – CO_2, 20); ^1H NMR ($CDCl_3$) δ 7.10 (b, 5 H, Ph), 5.10 (s, 2 H, CH_2Ph), 3.90 (s, 2 H, pyrr-CH_2-pyrr), 3.75, 3.55 (s, s, 4 H, CH_2CO), 3.50 (s, 12 H, OCH_3), 2.40–2.60 (m, 8 H, -CH_2CH_2-), 1.40 (s, 9 H, $(CH_3)_3$).

***tert*-Butyl 3,3′-Bis[β-(methoxycarbonyl)ethyl]-4,4′-bis[(methoxycarbonyl)methyl]dipyrrylmethane-5-carboxylate (16a).** Dipyrrylmethane **12a** (1.5 g) dissolved in 100 mL of ethanol was reduced with hydrogen at 50 psi during 2 h over 0.8 g of 10% Pd on charcoal. The solution was evaporated to dryness after filtration of the catalyst, the residue was dissolved in a small volume of 5% methanol in chloroform, and the solution was filtered through a TLC silica gel column (4 × 30 cm) using the same eluant. The fractions containing the dipyrrymethane acid were pooled and evaporated to dryness, and the residue was decarboxylated by heating at 205 °C during 2 min in vacuo. The residue was dissolved in a small volume of 2% methanol in benzene and was filtered through a TLC silica gel column (2 × 20 cm) using the same solvent as eluant. The fractions containing the dipyrrylmethane **16a** were pooled and evaporated to dryness; the oily residue (0.5 g, 41%) had the following: mass spectrum, *m/e* (relative intensity) 562 (M^+, 15), 505 (M^+ – isobutylene, 50); NMR ($CDCl_3$) δ 6.50 (s, 1 H, H-5′), 3.90–3.50 (b, 18 H, OCH_3, CH_2CO, pyrr-CH_2-pyrr), 3.45 (s, 2 H, CH_2CO), 2.25–290 (m, 8 H, CH_2CH_2), 1.50 (s, 9 H, $(CH_3)_3$).

***tert*-Butyl 3,3′-Bis[β-(methoxycarbonyl)ethyl]-4,4′-bis[(methoxycarbonyl)methyl]-5′-formyldipyrrylmethane-5-carboxylate (20a).** A solution of 500 mg of dipyrrylmethane **16a** in 0.6 mL of dimethylformamide was kept at 5 °C, and 0.6 mL of benzoyl chloride was added in one portion. The mixture was kept under moisture-exclusion conditions at 20 °C during 1 H. Ethyl ether (20 mL) was then added to the mixture and the solution was extracted with water (3 × 5 mL). The pooled water extracts were reextracted with ethyl ether (10 mL), and the traces of the latter were eliminated from the aqueous solution with a stream of nitrogen. The aqueous solution was adjusted to pH 8 with a 10% sodium carbonate solution and left during 20 h at 20 °C. The oily precipitate was extracted into chloroform (3 × 10 mL), the latter extracts were pooled and evaporated, and the residue was crystallized from ethanol–water: 320 mg (61%); mp 96–98 °C; NMR ($CDCl_3$) δ 9.50 (s, 1 H, CHO), 4.00 (s, 2 H, pyrr-CH_2-pyrr), 3.65–3.75 (b, 16 H, OCH_3), 2.40–2.75 (m, 8 H, CH_2CH_2), 1.50 (s, 9 H, $(CH_3)_3$). Anal. Calcd for $C_{29}H_{38}N_2O_{11}$: C, 58.98; H, 6.44; N, 4.74. Found: C, 58.91; H, 6.40; N, 4.72.

***tert*-Butyl 3,4′-bis[β-(methoxycarbonyl)ethyl]-4,3′-bis[(methoxycarbonyl)methyl]dipyrrylmethane-5-carboxylate (14a)** was obtained from **10a**[10b] (1.3 g) following the procedures described for the synthesis of **16a**; 500 mg (47% overall yield) of **14a** were obtained: mass spectrum, *m/e* (relative intensity) 562 (M^+, 20); NMR ($CDCl_3$) δ 6.40 (s, 1 H, H-5′), 3.60–3.85 (b, 16 H, OCH_3, CH_2CO, pyrr-CH_2-pyrr), 3.50 (s, 2 H, CH_2CO), 2.50–2.75 (m, 8 H, CH_2CH_2), 1.50 (s, 9 H, $(CH_3)_3$).

***tert*-Butyl 3,4′-bis[β-(methoxycarbonyl)ethyl]-4,3′-bis[(methoxycarbonyl)methyl]-5′-formyldipyrrylmethane-5-carboxylate (18a)** was obtained from 500 mg of **14a** following the procedure described for the synthesis of **20a**; 360 mg (68%) of **19a** were obtained: mp 128–129 °C (ethanol); NMR ($CDCl_3$) δ 9.55 (s, 1 H, CHO), 3.95 (s, 2 H, pyrr-CH_2-pyrr), 3.50–3.80 (b, 16 H, OCH_3, CH_2O), 2.50–3.10 (m, 8 H, CH_2CH_2), 1.55 (s, 9 H, $(CH_3)_3$). Anal. Calcd for $C_{29}H_{38}N_2O_{11}$: C, 58.98; H, 6.44; N, 4.74. Found: C, 59.00; H, 6.50; N, 4.70.

***tert*-Butyl 3,3′-dimethyl-4,4′-bis[β-(methoxycarbonyl)ethyl]-5′-[(benzyloxy)carbonyl]dipyrrylmethane-5-carboxylate (9b)** was obtained from the reaction of 1.8 g of the acetate **3**[10c] with 1.3 g of pyrrole **7**[13] following the procedure described for the synthesis of **12a**; 2.6 g (92%) of oily **9b** were obtained: mass spectrum, *m/e* (relative intensity) 580 (M^+, 28), 179 (37); NMR ($CDCl_3$) δ 7.38 (s, 5 H, Ph), 5.25 (s, 2 H, CH_2Ph), 3.81 (s, 2 H, pyrr-CH_2-pyrr), 3.63 (s, 6 H, OCH_3), 2.99 (m, 4 H, CH_2CH_2CO), 2.48 (m, 4 H, CH_2CO), 1.98 (s, 6 H, CH_3) 1.59 (s, 9 H, $(CH_3)_3$).

***tert*-Butyl 3,3′-dimethyl-4,4′-bis[β-(methoxycarbonyl)ethyl]dipyrrylmethane-5-carboxylate (13b)** was obtained from **9b** (2.4 g) following the procedures described for the preparation of **16a**; 0.96 g (50% overall yield) of oily **13b** were obtained: mass spectrum, *m/e* (relative intensity) 446 (M^+, 49), 180 (61); NMR ($CDCl_3$) δ 6.40 (b, 1 H, H-5′), 3.81 (s, 2 H, pyrr-CH_2-pyrr), 3.74, 3.76 (s, 6 H, OCH_3), 2.76 (m, 8 H, CH_2CH_2CO), 2.11 (s, 6 H, CH_3), 1.58 (s, 9 H, $(CH_3)_3$).

***tert*-Butyl 3,3′-dimethyl-4,4′-bis[β-(methoxycarbonyl)ethyl]-5′-formyldipyrrylmethane-5-carboxylate (17b)** was obtained from **13b** (0.8 g) following the procedure described for **20a**; 0.57 g (65%) of **17b** were obtained: mp 114–116 °C (ethanol–water); NMR ($CDCl_3$) δ 9.54 (s, 1 H, CHO), 3.88 (s, 2 H, pyrr-CH_2-pyrr), 3.71, 3.69 (s, s, 6 H, OCH_3), 2.97 (m, 4 H, CH_2CH_2CO), 2.57 (m, 4 H, CH_2CO), 2.10, 2.05 (s, 6 H, CH_3), 1.57 (s, 9 H, $(CH_3)_3$). Anal. Calcd for $C_{25}H_{34}N_2O_7$: C, 63.29; H, 7.17; N, 5.91. Found: C, 63.30; H, 7.20; N, 5.80.

***tert*-Butyl 3,4′-bis[β-(methoxycarbonyl)ethyl]-4,3′-dimethyl-5′-[(benzyloxy)carbonyl]dipyrrylmethane-5-carboxylate (10b)** was obtained by condensation of 1.3 g of acetate **3** with 0.9 g of pyrrole **8**[13] (as its methyl ester) following the procedure described for the synthesis of **12a**; 1.6 g (85%) of oily **10b** were obtained: mass spectrum, *m/e* (relative intensity) 580 (M^+, 6), 314 (5); NMR ($CDCl_3$) δ 7.37 (s, 5 H, Ph), 5.29 (s, 2 H, CH_2), 3.92 (s, 2 H, pyrr-CH_2-pyrr), 3.68 (s, 6 H, OCH_3), 3.04 (m, 4 H, CH_2CH_2CO), 2.59 (m, 4 H, CH_2CO), 2.28 (s, 3 H, 4′-CH_3), 2.08 (s, 3 H, 3-CH_3), 1.58 (s, 9 H, $(CH_3)_3$).

***tert*-Butyl 3,4′-bis[β-(methoxycarbonyl)ethyl]-4,3′-dimethyldipyrrylmethane-5-carboxylate (14b)** was obtained from **10b** (1 g) following the procedures described for the synthesis of **16a**: 0.4 g (47% overall yield); mass spectrum, *m/e* (relative intensity) 446 (M^+, 95), 180 (80); NMR ($CDCl_3$) δ 8.33, 7.95 (b, 1 H, NH), 6.40 (b, 1 H, H-5′), 3.87 (pyrr-CH_2-pyrr), 3.80, 3.74 (s, 6 H, OCH_3), 2.60 (m, 8 H, CH_2CH_2) 2.30 (s, 3 H, 4-CH_3), 2.08 (s, 3 H, 3′-CH_3), 1.59 (s, 9 H, $(CH_3)_3$).

***tert*-Butyl 3,3′-bis[β-(ethoxycarbonyl)ethyl]-4,4′-dimethyl-5′-[(benzyloxy)carbonyl]dipyrrylmethane-5-carboxylate (12b)** was obtained from the reaction of 1 g of **8**[13] and 1.4 g of **4**[10c] following the procedure described for the synthesis of **12a**; 1.3 g (60%) of **12b** were obtained: mass spectrum, *m/e* (relative intens ity) 608 (M^+, 8), 551 (M^+ – isobutylene, 24), 461 (551 – CH_2Ph, 100); NMR ($CDCl_3$) δ 7.35 (s, 5 H, Ph), 5.20 (s, 2 H, CH_2Ph), 4.25–3.90 (b, 6 H, OCH_2CH_3), 2.65–2.40 (m, 8 H, CH_2CH_2), 2.65–2.20 (s, s, 6 H, CH_3), 1.55 (s, 9 H, $(CH_3)_3$), 1.20, 1.15 (t, t, 6 H, OCH_2CH_3).

***tert*-Butyl 3,3′-bis[β-(ethoxycarbonyl)methyl]-4,4′-dimethyldipyrrylmethane-5-carboxylate (16b)** was obtained from **12b** (1.3 g) following the procedures described for the preparation of **16a**; 0.6 g (65% overall yield) of **16b** were obtained: mass spectrum, *m/e* (relative intensity) 474 (M^+, 25); NMR ($CDCl_3$) δ 6.35 (s, 1 H, H-5′), 4.25–3.90 (m, 4 H, OCH_2CH_3), 3.85 (s, 2 H, pyrr-CH_3-pyrr), 2.75–2.45 (m, CH_2CH_2), 2.25 (s, 3 H, CH_3), 2.0 (s, 3 H, CH_3), 1.50 (s, 9 H, $(CH_3)_3$), 1.40 (t, t, 6 H, OCH_2CH_3).

***tert*-Butyl 3,3′-bis[β-(ethoxycarbonyl)methyl]-4,4′-dimethyl-5′-formyldipyrrylmethane-5-carboxylate (20b)** was obtained from **16b** (0.6 g) following the procedure described for **20a**; 0.41 g (65%) of **20b** were obtained: mp 120–122 °C (ethanol–water); NMR ($CDCl_3$) δ 9.50 (s, 1 H, CHO), 4.25–4.0 (m, 6 H, OCH_2CH_3, pyrr-CH_2-pyrr), 2.85–2.45 (m, 8 H, CH_2CH_2), 2.35–2.30 (s, s, 6 H, CH_3), 1.55 (s, 9 H, $(CH_3)_3$), 1.25 (t, t, 6 H, OCH_2CH_3). Anal. Calcd for $C_{25}H_{34}N_2O_7$: C, 63.29; H, 7.17; N, 5.91. Found: C, 63.25; H, 7.10; N, 5.60.

Urobiliverdin I (Bactobilin) Octamethyl Ester. A mixture of 97 mg (0.16 mmol) of aldehyde **19a**[10b] and 90 mg (0.16 mmol) of dipyrrylmethane **14a** was dissolved in 3 mL of anhydrous methanol and 0.3 mL of 48% HBr was added. The solution was kept at 20 °C during 15 min and was then poured over a column (1.5 × 20 cm) of deactivated alumina (prepared by suspending Merck grade I alumina in methanol, filtering, and drying in air), prewashed with chloroform. The bilene **21a** (yellow band) was eluted with the same solvent and was collected in a mixture of methanol and 48% HBr. The eluates were evaporated to dryness

in vacuo and the red residue was redissolved in dry ethyl ether and the solution was again evaporated to dryness. The hydrobromide **21** [NMR ($CDCl_3$) δ 7.50 (b, 1 H, CH=), 5.00 (b, 4 H, pyrr-CH_2-pyrr), 3.87, 3.75 (s, s, 4 H, CH_2CO), 3.69, 3.68 (b, b, 24 H, OCH_3), 3.34, 3.28 (s, s, 4 H, CH_2CO), 2.56 (b, 16 H, CH_2CH_2CO), 1.55 (s, 18 H, $(CH_3)_3$)] was dissolved in 30 mL of trifluoroacetic acid previously flushed with nitrogen, and the mixture was kept at 20 °C during 15 min under a stream of nitrogen. Bromine (0.03 mL) was then added in three portions over a 1-h period, while the solution was kept at −10 °C. The mixture was then poured over degassed water (100 mL), the aqueous solution was extracted with chloroform (4 × 30 mL), and the chloroform extracts were washed with 8% sodium bicarbonate, dried (Na_2SO_4), and evaporated to dryness. The residue was disolved in a mixture of chloroform/acetone/methanol (94:4:2) and was filtered through a TLC silica gel column (4 × 30 cm). The fractions containing the blue band were pooled and evaporated to dryness, and the residue was crystallized from chloroform–hexane: 12 mg (8%); mp 178–180 °C; mass spectrum, *m/e* (relative intensity) 960 (M^+ − 2, 1), 488 (dipyrromethenone half plus CH, 4), 476 (dipyrromethenone half, 4), 456 (M^+ − 474 − 30 ($2CH_3$), 15), 444 (M^+ − 488 − 30 ($2CH_3$), 19), 400 (444 − 44 (CO_2), 9), 386 (400− 14 (CH_3), 13), 371 (458 −87 ($CH_2CH_2CO_2CH_3$), 10). Anal. Calcd for $C_{47}H_{54}N_4O_{18}$: C, 58.62; H, 5.61; N, 5.82. Found: C, 58.39; H, 5.50; N, 5.72.

Coprobiliverdin I tetramethyl ester was obtained following the procedure described above. By condensation of 90 mg of the aldehyde **19b**[10c] (as its diethyl ester) and 80 mg of **14b**, the b-bilene **21b** was obtained as its dimethyl and diethyl ester and was oxidized to coprobiliverdin I as described above. After filtration through the TLC silica gel column using chloroform/acetone/methanol (100:4:2) as elution solvent, the obtained coprobiliverdin I ester was dissolved in 5 mL of 5% sulfuric acid in methanol and kept at 20 °C during 18 h. The solution was diluted with chlorform and then washed with a 8% sodium bicarbonate solution, and the organic layer was evaporated to dryness in vacuo. The residue of coprobiliverdin tetramethyl ester was crystallized from chloroform–benzene: 15 mg (11%); mp 188–190 °C; NMR ($CDCl_3$) δ 6.60 (s, 1 H, H-10), 5.80 (s, 2 H, H-5 and H-15), 3.70 (s, s, s, 12 H, OCH_3), 2.80 (m, 6 H, CH_2-3a, 8a, and 13a), 2.75 (m, 6 H, CH_2-3b, 8b, 13b, 18a, and 18b), 2.20, 2.10, 2.05 (s, s, s, 9 H, CH_3-7, 12, and 17), 1.80 (s, 3 H, CH_3-2). Anal. Calcd for $C_{39}H_{46}N_4O_{10}$: C, 64.09; H, 6.34; N, 7.66. Found: C, 64.10; H, 6.30; N, 7.56.

Urobiliverdin IIIα octamethyl ester was obtained following the described procedure by condensation of **18a** (65 mg) and **13a**[10a] (60 mg); 8 mg (8%) of urobiliverdin IIIα octamethyl ester were obtained by oxidation of the b-bilene hydrobromide **22a**: mp 185–187 °C (chloroform–hexane); mass spectrum, *m/e* (relative intensity) 960 (M^+ − 2, 3). Anal. Calcd for $C_{47}H_{54}N_4O_{18}$: C, 58.62; H, 5.61; N, 5.82. Found: C, 58.81; H, 5.82; N, 5.90.

Coprobiliverdin IIIα tetramethyl ester was obtained by condensation of **17b** (75 mg) and **14b** (70 mg) to give the b-bilene hydrobromide **22b** which was then oxidized to the coprobiliverdin IIIα tetramethyl ester: 13 mg (12%); mp 190–192 °C (chloroform–hexane); mass spectrum, *m/e* (relative intensity) 730 (M^+, 16); NMR ($CDCl_3$) δ 6.70 (s, H, C-10), 5.85, 5.80 (s, s, 2H, C-5 and C-15), 3.70, 3.75 (s, s, 12 H, OCH_3), 2.80, 2.60 (t, t, 8 H, CH_2-3a, 8a, 12a, 18a), 2.50 (b, 8 H, CH_2-3b, 8b, 12b, 18b), 2.10, 2.05 (s, b, 9 H, CH_3-7, 12, 17), 1.80 (s, 3 H, CH_3-2). Anal. Calcd for $C_{39}H_{46}N_4O_{10}$: C, 64.09; H, 6.34; N, 7.66. Found: C, 64.05; H, 6.25; N, 7.76.

Urobiliverdin IIIβ octamethyl ester was obtained by condensation of the formyldipyrrylmethane **20a** (100 mg) and **15a**[10b] (90 mg) to give the b-bilene hydrobromide **23a** which was oxidized to the urobiliverdin ester was described above: 15 mg (9%); mp 183–185 °C (chloroform–hexane); mass spectrum, *m/e* (relative intensity) 960 (M^+ − 2, 2). Anal. Calcd for $C_{47}H_{54}N_4O_{18}$: C, 58.62; H, 5.61; N, 5.82. Found C, 58.65; H, 5.73; N, 5.78.

Coprobiliverdin IIIβ tetramethyl ester was obtained by condensation of the dipyrrylmethanes **19b**[10c] (90 mg) (as its diethyl ester) and **16b** (80 mg) to give **23b** (as its ethyl ester) which was oxidized to the corresponding biliverdin and transesterified with 5% sulfuric acid in methanol to give coprobiliverdin IIIβ octamethyl ester: 12 mg (10%); mp 135–137 °C (chloroform–hexane); mass spectrum, *m/e* (relative intensity) 730 (M^+, 19), 360 (100); NMR ($CDCl_3$) δ 6.60 (s, 1 H, H-10), 5.85, 5.80 (s, s, 2 H, C-5 and C-15); 3.75, 3.70 (b, b, 12 H, OCH_3) 2.75 (m, 6 H, CH_2-3a, 7a, 13a), 2.55 (b, 10 H, CH_2-3b, 7b, 13b, 18b, 18a), 2.15, 2.10 (s, s, 9 H, CH_3-8, 12, 17), 1.80 (s, 3 H, CH_3-2). Anal. Calcd for $C_{39}H_{46}N_4O_{10}$: C, 64.09; H, 6.34; N, 7.66. Found: C, 64.15; H, 6.21; N, 7.50.

Urobiliverdin IIIγ octamethyl ester was obtained by condensation of the formyldipyrrylmethane **17a**[10a] (90 mg) and the dipyrrylmethane **15a**[10b] (80 mg) to give the b-bilene hydrobromide **24a** which was oxidized to the urobiliverdin: 8 mg (5%); mp 203–205 °C (chloroform–hexane); mass spectrum, *m/e* (relative intensity) 960 (M^+ − 2, 5). Anal. Calcd for $C_{47}H_{54}N_4O_{18}$: C, 58.62; H, 5.61; N, 5.82. Found: C, 58.85; H, 5.50; N, 5.70.

Coprobiliverdin IIIγ tetramethyl ester was obtained by condensation of **17b** (72 mg) and the diethyl ester of **15b**[10c] (70 mg) to give the b-bilene hydrobromide **24b** which was oxidized to the biliverdin and then transesterified to the tetramethyl ester: 11 mg (10%); mp 202–203 °C; mass spectrum, *m/e* (relative intensity) 730 (M^+, 18); NMR ($CDCl_3$) δ 6.57 (s, 1 H, H-10), 5.82, 5.77 (s, s, 2 H, C-5 and C-15), 3.67, 3.65 (b, b, 12 H, OCH_3), 2.75 (m, 4 H, CH_2-8a, 13a), 2.55 (b, 12 H, CH_2-2a, 18a, 2b, 8b, 13b, 18b), 2.15, 2.10, 2.05 (s, s, s, 12 H, CH_3). Anal. Calcd for $C_{39}H_{46}N_4O_{10}$: C, 64.09; H, 6.34; N, 7.66. Found: C, 64.10; H, 6.20; N, 7.65.

Urobiliverdin IIIδ octamethyl ester was obtained by condensation of the formyldipyrrylmethane **20a** (100 mg) and the dipyrrylmethane **14a** (90 mg) to give the b-bilene hydrobromide **25a** which was oxidized to the urobiliverdin IIIδ octamethyl ester; 13 mg (7.5%) of the noncrystalline isomer was obtained: mass spectrum, *m/e* (relative intensit) 962 (M^+, 6), 476 (8), 488 (9), 444 (20).

Coprobiliverdin IIIδ tetramethyl ester was obtained by condensation of the dipyrrylmethane **20b** (90 mg) and **14b** (80 mg) to give the b-bilene hydrobromide **25b** which was oxidized to the biliverdin and the latter than transesterified to finally give coprobiliverdin IIIδ tetramethyl ester: 20 mg (16%); mp 92–94 °C (chloroform–hexane); mass spectrum, *m/e* (relative intensity) 730 (M^+, 20); NMR ($CDCl_3$) δ 6.50 (s, 1 H, H-10), 6.00, 5.95 (s, s, 2 H, H-5 and H-15), 3.65, 3.70 (s, s, 12 H, OCH_3), 2.95–2.50 (m, 16 H, -CH_2CH_2-), 2.20, 2.10 (s, s, 6 H, CH_3-7, 12), 1.80 (b, 6 H, CH_3-2, 18). Anal. Calcd for $C_{39}H_{46}N_4O_{10}$: C, 64.09; H, 6.34; N, 7.66. Found: C, 64.13; H, 6.31; N, 7.56.

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Registry No. **2**, 51912-05-1; **3**, 51671-81-9; **4**, 51741-18-5; **5**, 75975-52-9; **7**, 42419-19-2; **8** (ethyl ester), 102613-70-7; **8** (methyl ester), 51644-18-9; **9b**, 102613-66-1; **10a**, 70988-57-7; **10b**, 74839-16-0; **12** (acid), 98813-90-2; **12a**, 102613-65-0; **12b** (diethyl ester), 102613-71-8; **13a**, 75975-55-2; **13b**, 102613-67-2; **14a**, 84315-34-4; **14b**, 102613-69-4; **15a**, 96246-53-6; **15b** (diethyl ester), 68813-12-7; **16a**, 96246-53-6; **16b** (diethyl ester), 102613-72-9; **17a**, 75975-56-3; **17b**, 102613-68-3; **18a**, 84315-35-5; **19a**, 84315-36-6; **19b** (diethyl ester), 68781-41-9; **20a**, 84315-36-6; **20b** (diethyl ester), 102613-73-0; **21a**, 96246-54-7; **21b** (diethyl ester), 102613-74-1; **22a**, 96246-55-8; **22b**, 102613-77-4; **23a**, 96246-56-9; **23b** (tetraethyl ester), 102613-79-6; **24a**, 75993-24-7; **24b** (diethyl ester), 102613-81-0; **25a**, 96246-57-0; **25b** (diethyl ester), 102613-84-3; I (R = $CH_2CO_2CH_3$), 96246-52-5; I (R = CH_3) (diethyl ester), 102613-75-2; I (R = CH_3), 102613-76-3; IIIα (R = $CH_2CO_2CH_3$), 88815-22-9; IIIα (R = CH_3), 102613-78-5; IIIβ (R = $CH_2CO_2CH_3$), 88815-23-0; IIIβ (R = CH_3) (tetraethyl ester), 102629-96-9; IIIβ (R = CH_3), 102613-80-9; IIIγ (R = $CH_2CO_2CH_3$), 88815-25-2; IIIγ (R = CH_3) (diethyl ester), 102613-82-1; IIIγ (R = CH_3), 102613-83-2; IIIδ (R = $CH_2CO_2CH_3$), 88815-25-2; IIIδ (R = CH_3) (diethyl ester), 102613-85-4; IIIδ (R = CH_3), 102613-86-5; bactobilin, 96246-58-1.