Tetrahedron Vol 39, No 11, pp 1841 to 1847, 1983
Printed in Great Britain

0040-4020/83/111841-07$03.00/0
Pergamon Press Ltd

BILE PIGMENT STUDIES—VI[1]

SYNTHESES OF MODEL SYSTEMS

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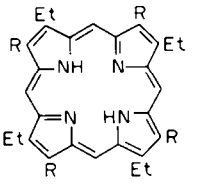
(*Received in U.K. 10 June 1982*)

Abstract—The synthesis of various mammalian and algal bile pigment models from monopyrroles and by ring cleavage of intact metalloporphyrins and metallochlorins is discussed. A previously reported synthesis of etiobiliverdin IVγ (**6**) from the self-condensation of a 5-bromo-5′-bromomethyldipyrrylmethene hydrobromide (**5**) is modified to afford a new, efficient and general route to biliverdins through 1,19-di-t-butoxycarbonyl-*a,c*-biladienes or -*b*-bilenes. Owing to symmetry limitations inherent in the *a,c*-biladiene route, that through *b*-bilenes is shown to be more generally effective for the synthesis of biliverdins. The key step in the transformation of the biladiene or bilene into biliverdin involves treatment with bromine in trifluoroacetic acid, and this affords biliverdin in high yield. The route is proposed to proceed through a 1,19-dibromo-*a,b,c*-bilitriene and then a 1,19-di-(trifluoroacetoxy)-*a,b,c*-bilitriene, though these intermediates are not isolated.

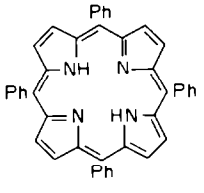
INTRODUCTION

RESEARCH in the porphyrin and chlorophyll areas has been substantially aided by the availability of model systems,[2] these usually being derivatives of either octaethylporphyrin[3,4] (**1**) or tetraphenylporphyrin[5,6] (**2**). In contrast, bile pigment model compounds have not been generally accessible, and so a systematic study of biliverdin and bilirubin chemistry has been held back by lack of suitable materials. Hans Fischer did describe several straightforward routes to model bile pigment systems,[7] but they have never been exploited in a manner comparable with the situation for porphyrin systems. Indeed, for a long while, model bile pigments such as octaethylbiliverdin (**3**) were produced by coupled oxidation of octaethylhemin,[8,9] rather than by a rational synthesis, though one has very recently been described.[10] While synthesis of bile pigments from porphyrins has definite advantages, particularly when unsymmetrically substituted porphyrins can be used to give a separable mixture of four identifiable isomers,[11,12] it can never provide the large quantities of pigments required to set bile pigment chemistry on the firm foundation enjoyed by porphyrin and metalloporphyrin systems.

In Part I[13] of this series, we described the accidental discovery that during the course of a synthesis of etioporphyrin I (**4**) by head-to-tail self-condensation of the dipyrrylmethene hydrobromide (**5**) in formic acid, a certain amount of tail-to-tail self-condensation, with concomitant loss of a C atom, took place to give etiobiliverdin IVγ (**6**). Minor modification of the formic acid reaction to include 10% water finally gave a yield of 22% of the required verdin (**6**) with only 13% of etioporphyrin I (**4**).



1 R = Et
4 R = Me



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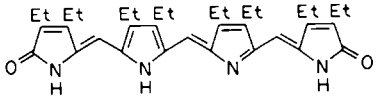

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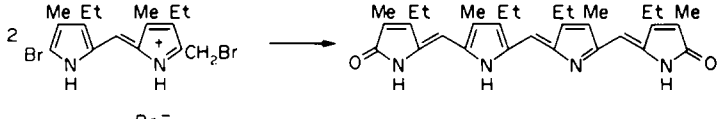
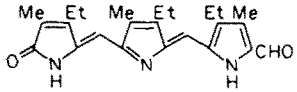


5 **6**

Ready availability of **6** spurred Falk *et al.* to study the physical-organic chemistry of biliverdins (e.g. ref. 14), and these workers also developed a slightly different synthetic approach to **6** which avoided formation of the porphyrin contaminant.[15]

Part III[16] described reactions of etiobiliverdin IVγ (**6**) with thallium(III) acetate in methanol, from which the formyltripyrrinone (**7**), ethylmethylmaleimide (**8**), the 5-ketobilindione (**9**), and the 4,5-dimethoxybilindione (**10**) were isolated. The reactions were shown to be facilitated by formation of the metallobilindione cation-radical (**11**), followed by nucleophilic attack of either acetate or methanol.[16] The structures of both the formyltripyrrinone (**7**)[17] and the 4,5-dimethoxybilindione (**10**)[18] have subsequently been confirmed by single crystal X-ray studies.

Realizing that the key to biliverdin oxidative chemistry lay in the initial formation of a cation-radical by one electron abstraction, a cyclic voltammetric study was carried out[19] using etiobiliverdin IVγ (**6**) as the substrate, and this revealed three oxidation waves; the first and second of these (at *ca* 300 mV and 1.1 V *vs* s.c.e. at Pt electrodes) were



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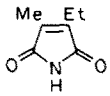

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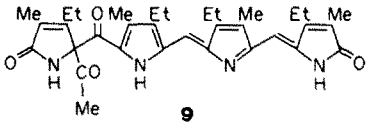

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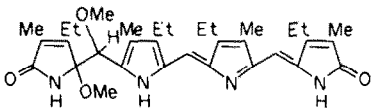

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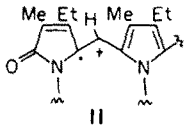

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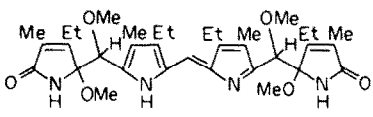

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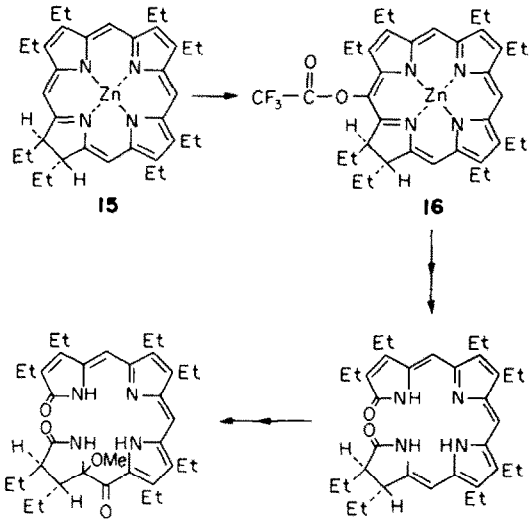
clearly reversible and were interpreted to correspond to formation of the cation-radical and dication of the bilindione (**6**). Preparative electrolysis of **6** in methylene chloride containing methanol, at 0.7 V (*vs* s.c.e. at a Pt mesh) gave a 73% yield of the 4,5-dimethoxybilindione (**10**). At higher potentials the tetramethoxy derivative (**12**) was isolated.[1]

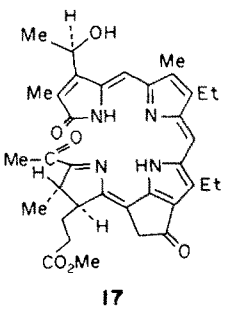
In a separate study, Part II[20] described the syntheses of the phytochrome models (**13** and **14**) by ring cleavage of zinc(II) *trans*-octaethylchlorin (**15**) using thallium(III) trifluoroacetate to accomplish initial *meso*-trifluoroacetoxylation of the nucleus.[21] The resulting *meso*-trifluoroacetoxychlorin (**16**) (Scheme 1) was hydrolyzed and reacted with oxygen to give **13** which, *in situ*, suffered further oxidation and functionalization to give **14**. The 2,3-dihydrobilindione chromophore (as in **13**) has assumed great importance in recent times because of its relationship to the proposed structure[22] of the phytochrome chromphore, and Scheer in particular has carried out several further oxidative and nucleophilic substitution studies on **13**.[23]



15 **16** **14** **13**

Scheme 1.

Other examples of model bile pigment systems have been obtained by ring cleavage of chlorin systems, and perhaps the best characterized of these are the acetylbilinones (**17**) obtained by photo-oxygenation of derivatives of the bacteriochlorophylls-*c*.[24-29] Again, the overall chromophore could be regarded as a model for the phytochrome system (because it has the 2,3-dihydro system), but such chlorophyll degradation products might also be important with regard to species produced in the degradation of natural chlorophylls in senescent leaves, and in fruit ripening. A similar pigment with a benzoyl group (e.g. **18**) in place of the acetyl has been obtained by treatment of zinc(II) tetraphenylporphyrin with thallium(III) salts,[30] or by photo-oxygenation of metallotetraphenylporphyrins,[31,32] and these pigments closely



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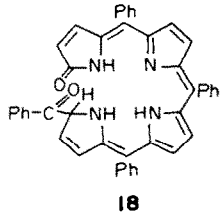

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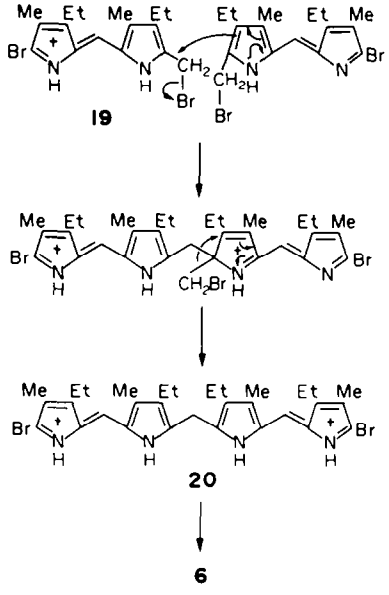
resemble the formylbilitrienes produced by Fuhrhop *et al.*[33-35] by photo-oxygenation of metallo-porphyrins and metallochlorins.

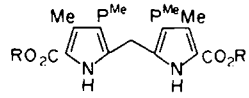
Development of a general synthetic approach to bilindiones (biliverdins)

A plausible mechanism for the formation of etiobiliverdin IV$_\gamma$ (**6**) from the bromobromomethyldipyrrylmethene hydrobromide (**19**)[36] in hot formic acid is shown in Scheme 2. The first step, in which the initial condensation occurs to give **20**, is reminiscent of the preparation of symmetrically substituted dipyrrylmethanes by heating bromomethylpyrroles in methanol.[37] The resulting 1,19-dibromo-*a,c*-biladiene dihydrobromide (**20**) would be expected to be oxidized in the hot formic acid,[38] and hydrolysis of the terminal bromo-functions to afford lactams would eventually give **6**. On the basis of this mechanism we supposed that 1,19-dibromo-*a,c*-biladiene salts (e.g. **20**) should be good intermediates for the synthesis of biliverdins.

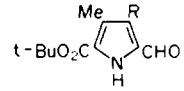


Scheme 2.

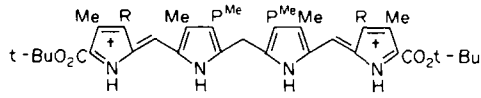
Thus, the dibenzyl dipyrrylmethane-5,5′-dicarboxylate (**21**) was hydrogenated over Pd-C and gave a quantitative yield of the dicarboxylic acid **22**.[39] Treatment with two mole equivalents of the 2-formylpyrrole (**23**) using *p*-toluenesulphonic acid as catalyst[40] gave an 86% yield of the 1,19-di-t-butoxycarbonyl-*a,c*-biladiene dihydrobromide (**24**) after exchange of the *p*-toluenesulphonate counterion using HBr gas. Initial attempts to remove the t-butoxycarbonyl groups with trifluoroacetic acid, followed by stepwise bromination and then heating in aqueous formic acid[13] were not promising, so a much simpler procedure was adopted. Treatment of the *a,c*-biladiene (**24**) with a slight excess of bromine in trifluoroacetic acid gave, *in one step*, a 71% yield of mesobiliverdin XIIIα (**25**) after an alkaline work-up. It proved to be essential to carry out the bromine/trifluoroacetic acid reaction under N_2 in order to obtain a good yield of the biliverdin. If oxygen was allowed to be present, then the major product was purple in color and possessed a bilipurpurin type of electronic absorption spectrum, no doubt due to formation of the biliverdin cation-radical (with bromine as oxidant) and further reaction and fragmentation reactions with O_2.[16,19]



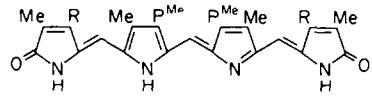
21 R = CH_2Ph
22 R = H



23 R = Et
29 R = P^{Me}



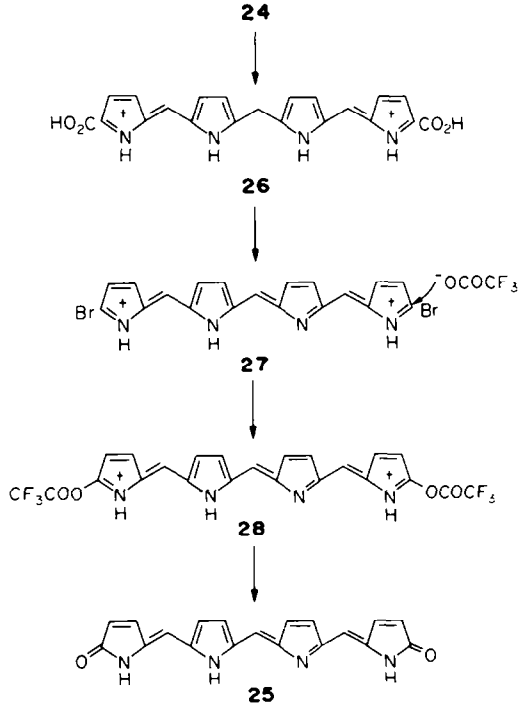
24 R = Et
30 R = P^{Me}



25 R = Et
31 R = P^{Me}

P^{Me} = $CH_2CH_2CO_2Me$

We surmise that the mechanistic pathway to the biliverdin (**25**) involves removal of the t-butyl esters by the trifluoroacetic acid to give the *a,c*-biladiene dicarboxylic acid (**26**) (Scheme 3) followed by oxidation to the bilitriene stage and bromination, to give **27**. Attack by trifluoroacetate upon **27** (to give **28**),



Scheme 3.

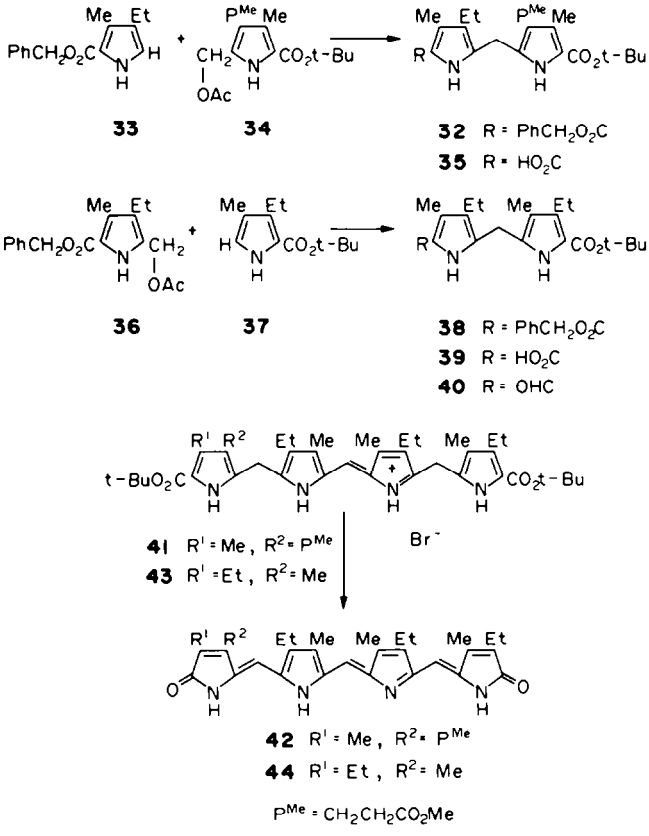
followed by cleavage under the alkaline conditions of the work-up, would then afford the required **25**.

The generality of this approach to biliverdins through *a,c*-biladienes was established by synthesis of a second example. Thus, the formylpyrrole (**29**) was condensed, with *p*-toluenesulphonic acid catalysis,[40] with the dipyrrylmethane dicarboxylic acid (**22**) to give an 84% yield of the *a,c*-biladiene dihydrobromide (**30**) after careful exchange of the counterion with HBr gas. With bromine in trifluoroacetic acid, under N_2, followed by an alkaline work-up, coprobiliverdin IVα tetramethyl ester (**31**) was obtained in 65% yield from **30**.

An obvious limitation to the general applicability of this route to the synthesis of biliverdins is that a symmetrical biliverdin must result, owing to the fact that the *a,c*-biladiene is prepared from a dipyrrylmethane and *two* mole equivs of a formylpyrrole. Asymmetry can be introduced into the *a,c*-biladiene by use of a dipyrrylmethane bearing an unsymmetrical array of substituents, but the fact that the majority of bile pigments of natural origin bear a symmetrical arrangement in the central rings [e.g. Me, P-P, Me (where P = $CH_2CH_2CO_2H$)] makes this variation of little advantage in a majority of cases. On the other hand, *a,c*-biladienes for use in porphyrin synthesis can be made unsymmetrical by the intermediacy of a so-called "tripyrrene".[41] However, such porphyrin precursors possess Me groups (usually) at the 1 and 19 positions in the *a,c*-biladiene and these tetrapyrroles are synthesized from a 1-t-butoxycarbonyltripyrrene using a 2-formyl-5-methylpyrrole in trifluoroacetic/hydrobromic acids. The chances that a 1,19-di-t-butoxycarbonyl-*a,c*-biladiene could be prepared under such acidic conditions are very slim, but alternative protection of the 1 and 19 positions which will allow such a stepwise approach to *a,c*-biladienes with terminal groups compatible with subsequent transformation into bile pigments are under investigation.

Symmetry limitations to the *a,c*-biladiene route (*vide supra*) can be very readily surmounted by the alternative use of *b*-bilenes; such compounds can be prepared with a totally unsymmetrical array of substituents using a formyldipyrrylmethane and a dipyrrylmethane-5-carboxylic acid.[40] In order to demonstrate this more general approach to bile pigments, the dipyrrylmethane (**32**) was synthesized from benzyl 4-ethyl-3-methylpyrrole-2-carboxylate (**33**) and t-butyl 5-acetoxymethyl-4-(2-methoxycarbonylethyl)-3-methylpyrrole-2-carboxylate (**34**) by heating in acetic acid containing a catalytic amount of *p*-toluenesulphonic acid.[42] Catalytic debenzylation gave the dipyrrylmethane-5-carboxylic acid (**35**). Meanwhile, condensation[42] of benzyl 5-acetoxymethyl-4-ethyl-3-methylpyrrole-2-carboxylate (**36**) with t-butyl 3-ethyl-4-methylpyrrole-2-carboxylate (**37**) gave the dipyrrylmethane (**38**) which was catalytically debenzylated (to give **39**) and then formylated under the Vilsmeier conditions to give **40**. Condensation of the dipyrrylmethanes (**35** and **40**) in the presence of *p*-toluenesulphonic acid[40] gave an 88% yield of the *b*-bilene hydrobromide (**41**) after counterion exchange using HBr gas. Treatment with a slight excess of bromine in trifluoroacetic acid, under N_2, then afforded a 76% yield of the unsymmetrically substituted biliverdin methyl ester **42**.

In a similar sequence of reactions, the dipyrrylmethane-5-carboxylic acid (**35**) was condensed with



the 5-formyldipyrrylmethane (**40**) to give the *b*-bilene hydrobromide **43** in 92% yield, and this was transformed into etiobiliverdin IVα (**44**), as described above, in a yield of 73%

EXPERIMENTAL

Mp.s were measured on a hot-stage apparatus and are uncorrected. Neutral alumina (Merck; Brockmann Grade III) was used for column chromatography and reactions were routinely monitored using Merck silicagel GF-254 precoated sheets (0.2 mm). Preparative thick layer chromatography was carried out on 20 × 20 cm glass plates coated with Merck GF-354 silicagel (1.5 mm). Electronic absorption spectra were determined using a Hewlett-Packard 8450 spectrophotometer (solns in CH_2Cl_2), and ^1H NMR spectra were measured on either a Varian EM390 (90 MHz) or a Nicolet NT360 (360 MHz) spectrometer (solns in $CDCl_3$ with TMS as internal calibrant). Mass spectra (direct insertion probe, 70 eV, 50 μA, source temp *ca* 200°) were obtained using a Finnegan 3200 spectrometer. Elemental analyses were carried out at the UC Berkeley Microanalytical Laboratory.

Benzyl 5′-t-butoxycarbonyl-3,4′-diethyl-3′,4-dimethyldipyrrylmethane-5-carboxylate (**38**)

Compounds **37** (1.23 g) and **36** (1.85 g) in AcOH (50 ml) containing *p*-toluenesulphonic acid hydrate (55 mg) were stirred under N_2 for 4 hr at 40°. The mixture was cooled, diluted with water (100 ml) and CH_2Cl_2 (100 ml) and the organic phase was washed with more water (3 × 100 ml) and then with $NaHCO_3$aq (2 × 100 ml). The solvent was evaporated and the residue was chromatographed on alumina (elution with CH_2Cl_2); evaporation of the appropriate eluates (monitoring by TLC and exposing the developed plate to Br_2 vapor) gave a residue which was crystallized from CH_2Cl_2/n-hexane to give 1.5 g (55%) of the dipyrrylmethane, mp. 124 125°. (Found: C, 72.48; H, 7.66; N, 6.04. $C_{28}H_{36}N_2O_4$ requires: C, 72.39; H, 7.81; N, 6.03%). NMR, δ 1.00, 1.10 (each 3H, t, CH_3CH_2); 1.50 (9H, s, t-Bu); 1.92, 2.25 (each 3H, s, 3′,4-Me); 2.38, 2.65 (each 2H, q, CH_2CH_3); 3.78 (2H, s, $-CH_2-$); 5.23, 7.33 (2H, 5H, each s, CH_2Ph); 8.53, 8.76 (each 1H, br s, NH).

Benzyl 5′-t-butoxycarbonyl-3-ethyl-3′-(2-methoxycarbonylethyl-4,4′-dimethyldipyrrylmethane-5-carboxylate (**32**)

This dipyrrylmethane was prepared as above, using **33** and **34**. The product was recrystallized from CH_2Cl_2/n-hexane (70% yield) and had mp. 105° (lit.[43] mp. 104–105°).

5′-t-Butoxycarbonyl-3,4′-diethyl-3′,4-dimethyldipyrrylmethane-5-carboxylic acid (**39**)

Compound **38** (2 g) was dissolved in THF (100 ml) containing Et_3N (0.1 ml) and 10% PdC (200 mg) was hydrogenated at room temp and atmospheric pressure until uptake of H_2 ceased. The catalyst was removed by filtration through Celite and the solvent was evaporated to give a white residue which was crystallized from CH_2Cl_2/n-hexane to give 1.42 g (92%) of the dipyrrylmethane carboxylic acid, mp. 186–187° (dec). (Found: C, 67.40; H, 7.99; N, 7.47. $C_{21}H_{30}N_2O_4$ requires: C, 67.35: H, 8.08; N, 7.48%). NMR, δ, 1.00, 1.06 (each 3H, t, CH_3CH_2); 1.50 (9H, s, t-Bu); 2.05, 2.28 (each 3H, s, 3′, 4-Me); 2.40, 2.62 (each 2H, q, CH_2CH_3); 3.80 (2H, s, $-CH_2-$); NH's and CO_2H not observed.

5′-t-Butoxycarbonyl-3,4′-diethyl-5-formyl-3′,4-dimethyldipyrrylmethane (**40**)

The foregoing **39** (1 g) in CH_2Cl_2 (25 ml) was treated for 1 hr with a soln of *p*-toluenesulphonic acid hydrate (1 g) in MeOH (20 ml). CH_2Cl_2 (100 ml) was added and the soln was washed with water (3 × 100 ml) and then with $NaHCO_3$aq (2 × 100 ml). The organic phase was dried (Na_2SO_4) and evaporated to dryness to give the 5-unsubstituted dipyrrylmethane as an oil. Meanwhile, dry DMF (1.5 ml) was stirred in an ice bath (0–5°) and treated with phosphoryl chloride (1.9 ml), addition through a dropping funnel taking about 30 min. The crystalline Vilsmeier complex was dissolved in dry CH_2Cl_2 (10 ml) and then stirred for 15 min before being brought to room temp. The mixture was again cooled to 0–5° and then stirred with the 5-unsubstituted dipyrrylmethane in CH_2Cl_2 (10 ml) at room temp for 30 min before being heated under reflux for 1 hr. After cooling, the mixture was treated with excess satd $NaHCO_3$aq and was stirred overnight. The mixture was then refluxed for 1 hr, cooled, and the CH_2Cl_2 layer was separated. After drying (Na_2SO_4), the organic phase was evaporated to dryness to give an oil which was chromatographed on alumina (elution with EtOAc/cyclohexane, 1:9). The product was crystallized from MeOH to give 475 mg (50%) of formyldipyrrylmethane, mp. 149–150°. (Found: C, 70.59; H, 8.39; N, 7.85. $C_{21}H_{30}N_2O_3$ requires: C, 70.36; H, 8.44; N, 7.81%). NMR, δ, 1.06, 1.10 (each 3H, t, CH_3CH_2); 1.50 (9H, s, t-Bu), 2.00, 2.30 (each 3H, s, 3′, 4-Me); 2.45, 2.70 (each 2H, q, CH_2CH_3); 3.90 (2H, s, $-CH_2-$); 9.55 (1H, s, CHO); 9.68 (1H, br s, NH). second NH not observed.

5′-t-Butoxycarbonyl-3-ethyl-3′-(2-methoxycarbonylethyl)-4,4′-dimethyldipyrrylmethane-5-carboxylic acid (**35**)

This compound was prepared by hydrogenation of **32** as described for **39**. It was obtained in 95% yield and had mp. 91–93° (dec). (Found: C, 63.91; H, 7.50; N, 6.52. $C_{23}H_{32}N_2O_6$ requires: C, 63.87; H, 7.46; N, 6.48%). NMR, δ, 1.05 (3H, t, CH_3CH_2); 1.60 (9H, s, t-Bu); 2.22, 2.31 (each 3H, s, 4,4′-Me); 2.45–2.90 (6H, m, CH_2CH_3, CH_2CH_2CO); 3.70 (3H, s, OMe); 3.90 (2H, s, CH_2-); NH and CO_2H protons not observed.

Di-t-butyl 3,17-diethyl-8,12-di(2-methoxycarbonylethyl)-2,7,13,18-tetramethyl-a,c-biladiene-1,19-dicarboxylate dihydrobromide (**24**)

To a soln of **23** (237 mg; 1 mMole) and **22** (217 mg; 0.5 mMole) in CH_2Cl_2 (100 ml) was added a soln of *p*-toluenesulphonic acid hydrate (475 mg; 2.5 mMole) in dry MeOH (10 ml). The mixture was stirred under a dry atmosphere at room temp for 10 hr. The mixture was then poured into water (200 ml) and the organic phase was washed successively with satd $NaHCO_3$aq (2 × 50 ml) and water (2 × 50 ml). Evaporation of the organic solvent after drying, (Na_2SO_4), gave a semi-solid residue which was taken up in dry CH_2Cl_2 (20 ml) and treated with HBr gas for 5 sec. The solvent was *immediately* removed under vacuum and the residue was dissolved in dry benzene (20 ml), which was also evaporated quickly. This operation was repeated once more, and then anhyd ether (20 ml) was added and evaporated. After a further addition and removal of ether, the residue was crystallized from CH_2Cl_2/ether to give bright red microprisms (406 mg; 86%) of the *a,c*-biladiene, mp. >300°. (Found: C, 56.89; H, 6.58; N, 5.89. $C_{45}H_{60}N_4O_8 \cdot 2HBr$ requires: C, 57.08; H, 6.38; N, 5.91%). NMR, δ, 1.19 (6H, t, 2 × CH_2CH_3); 1.67 (18H, s, 2 × t-Bu); 2.27, 2.35 (each 6H, s, 2 × Me); 2.27, 2.73, 2.87 (m, q, t, each 4H, 2 × CH_2CH_3, 2 × CH_2CH_2CO); 3.50 (6H, s, 2 × OMe); 5.50 (2H, s, $-CH_2-$); 7.35 (2H, s, 2 × =CH−); 12.39, 14.89 (each 2H, br s, 2 × NH). λ_{max} 460 (ε 61,000) and 530 nm (43,000).

Di-t-butyl 3,8,12,17-tetra(2-methoxycarbonylethyl)-2,7,13,18-tetramethyl-a,c-biladiene-1,19-dicarboxylate dihydrobromide (**30**)

This compound was similarly prepared from **29** (295 mg; 1 mMole) and **22** (217 mg; 0.5 mMole). It was crystallized from CH_2Cl_2/ether and gave 415 mg (84%) of red-brown microprisms, mp. 170–175°. (Found: C, 55.43; H, 5.97; N, 5.51. $C_{49}H_{64}N_4O_{12} \cdot 2HBr$ requires: C, 55.37; H, 6.06; N, 5.26%). NMR, δ, 1.70 (18H, s, 2 × t-Bu); 2.30, 2.35 (each 6H, s, 2 × Me); 2.2–2.9 (16H, m, CH_2CH_2CO); 3.50, 3.55 (each 6H, s, OMe); 5.55 (2H, s, CH_2-); 7.37 (2H, s, −CH=); 12.00, 14.60 (each 2H, br s, NH). λ_{max} 460 (ε 63,500), 530 (44,000).

Di-t-butyl 7,13,18-*triethyl*-3-(2-*methoxycarbonylethyl*)-2,8,12,17-*tetramethylbilene*-b-1,19-*dicarboxylate hydrobromide* (**41**)

To a soln of **35** (216 mg; 0.5 mMole) and **40** (179 mg; 0.5 mMole) in CH_2Cl_2 (100 ml) was added a soln of *p*-toluenesulphonic acid hydrate (475 mg; 2.5 mMole) in MeOH (10 ml) and the mixture was stirred under anhyd conditions at room temp overnight. It was then washed with water (100 ml), satd $NaHCO_3$aq (2 × 50 ml), water again (2 × 100 ml) and then dried (Na_2SO_4) before being evaporated to dryness. Dry CH_2Cl_2 (20 ml) was added and then HBr gas was bubbled through the soln for 5 sec before rapid evaporation to dryness, addition and removal (rapidly, rotovapor) of dry benzene (50 ml, twice), and then addition and evaporation of ether. The resulting residue was crystallized from CH_2Cl_2/ether to give the *b*-bilene salt (357 mg; 88%) as bright red prisms, mp. 201–202°. (Found: C, 63.77; H, 7.80; N, 6.82. $C_{43}H_{61}N_4O_6 \cdot HBr$ requires: C, 63.69; H, 7.52; N, 6.90%). NMR, δ, 1.00 (9H, m, CH_2CH_3); 1.23 (18H, br s, t-Bu); 2.07, 2.10, 2.25, 2.30 (each 3H, s, Me); 2.4–2.9 (10H, m, CH_2CH_3, CH_2CH_2CO); 3.62 (3H, s, OMe); 4.30 (4H, s, $-CH_2-$); 7.15 (1H, s, $-CH=$); 10.40, 10.70, 13.70 (1H, 1H, 2H, each br s, NH). λ_{max} 504 nm (ε 65,000).

Di-t-butyl 2,7,13,18-*tetraethyl*-3,8,12,17-*tetramethylbilene*-b-1,19-*dicarboxylate hydrobromide* (**43**)

This compound was prepared as described above for **41**, using **39** (187 mg; 0.5 mMole) and **40** (179 mg; 0.5 mMole). The product was obtained as bright red prisms (344 mg; 92%) from CH_2Cl_2/ether and had mp. 101–103°. (Found: C, 65.60; H, 7.91; N, 7.28. $C_{41}H_{56}N_4O_4 \cdot HBr$ requires: C, 65.41; H, 7.83; N, 7.43%). NMR, δ 0.97, 1.03 (each 6H, t, CH_2CH_3); 1.50 (18H, s, t-Bu); 2.25, 2.29 (each 6H, s, Me); 2.40, 2.70 (each 4H, q, CH_2CH_3); 4.33 (4H, s, $-CH_2-$); 7.15 (1H, s, $CH=$); 10.37, 14.00 (each 2H, br s, NH). λ_{max} 502 nm (ε 66,000).

3,17-*Diethyl*-8,12-*di*(2-*methoxycarbonylethyl*)-2,7,13,18-*tetramethyl*-1,19-*bilindione* ("*Mesobiliverdin XIIIα*") (**25**)

Compound **24** (95 mg; 0.1 mMole) was stirred for 15 min at room temp under N_2 in nitrogen-purged trifluoroacetic acid (50 ml). Br_2 (0.03 ml) was then introduced using a microsyringe and the mixture was stirred with a brisk flow of N_2 gas passing through the mixture. After stirring for 1 hr the mixture was poured into nitrogen-purged distilled water (100 ml), was extracted with CH_2Cl_2 (3 × 100 ml), and then washed successively with water (100 ml), $NaHCO_3$aq (2 × 100 ml), and then water (100 ml). The organic phase was dried (Na_2SO_4), evaporated to dryness, and the resulting solid was chromatographed on alumina (elution with CH_2Cl_2). The blue eluates were collected, evaporated to dryness, and then crystallized from CH_2Cl_2/hexane to give the mesobiliverdin (44 mg; 71%) as dark blue needles, mp. 244–245° (lit.[44] mp. 246–247°). (Found: C, 68.15; H, 6.85; N, 9.00. Calc for $C_{35}H_{42}N_4O_6$: C, 63.38; H, 6.89; N, 9.11%). NMR, δ, 1.21 (6H, m, CH_3CH_2); 1.82 (6H, s, Me); 2.08 (6H, s, Me); 2.53 (8H, m, CH_2CH_2CO and CH_2CH_3); 2.92 (4H, t, CH_2CH_2CO); 3.67 (6H, s, OMe); 5.92 (2H, s, 5,15-H); 6.74 (1H, s, 10-H); 8.15 (3H, br s, NH). λ_{max} 372 (ε 68,200) and 642 nm (21,200). *m/e* (%), 614 (100), 315 (72), 302 (58), 234 (30).

3,8,12,17-*Tetra*-(2-*methoxycarbonylethyl*)-2,7,13,18-*tetramethyl*-1,19-*bilindione* ("*Coprobiliverdin IVα Tetramethyl Ester*") (**31**)

This compound was prepared as described above for **25**, from **30** (106 mg; 0.1 mMole). After crystallization from CH_2Cl_2/hexane, 48 mg (65%) of the biliverdin, mp. 176–177°, were obtained. (Found: C, 63.82; H, 6.30; N, 7.52. $C_{39}H_{46}N_4O_{10}$ requires: C, 64.09; H, 6.34; N, 7.66%). NMR, δ, 1.83, 2.10 (each 6H, s, Me); 2.55, 2.63, 2.83, 2.93 (each 4H, t, CH_2CH_2CO); 3.67, 3.70 (each 6H, s, OMe), 5.97 (2H, s, 5,15-H); 6.79 (1H, s, 10-H); 8.10 (3H, br s, NH). λ_{max} 378 (ε 56,100) and 646 (18,100). *m/e* (%), 730 (17), 360 (100), 287 (39).

7,13,18-*Triethyl*-3-(2-*methoxycarbonylethyl*)-2,8,12,17-*tetramethyl*-1,19-*bilindione* (**42**)

Compound **41** (162 mg; 0.2 mMole) was stirred at room temp for 15 min in nitrogen-purged trifluoroacetic acid (50 ml), and with N_2 passing through the mixture, it was cooled to 0° (ice bath) and Br_2 (0.05 ml) was introduced into the cooled soln through a microsyringe over a period of 1 hr. The mixture was then stirred for a further 1 hr at 0° with N_2 gas continuously passing through the mixture before it was poured into nitrogen-purged water (200 ml) and extracted with CH_2Cl_2 (3 × 100 ml). The organic phase was washed with water (100 ml), $NaHCO_3$aq (3 × 100 ml) and then once more with water before being dried (Na_2SO_4) and evaporated to dryness. The residue was chromatographed on alumina (elution with CH_2Cl_2) and the blue eluates were collected, evaporated to dryness and the resulting residue was crystallized from CH_2Cl_2/hexane to give the biliverdin (85 mg; 76%) as dark blue prisms, mp. 256–257°. (Found: C, 71.14; H, 7.29; N, 10.04. $C_{33}H_{40}N_4O_4$ requires: C, 71.20; H, 7.24; N, 10.06%). NMR, δ, 1.09 (9H, m, CH_3CH_2); 1.83, 2.09, 2.16 (3H, 3H, 6H, each s, Me), 2.26, 2.48 (2H, 4H, each q, CH_2CH_3); 2.64, 2.81 (each 2H, t, $COCH_2CH_2$); 3.71 (3H, s, OMe); 5.87, 5.92 (each 1H, s, 5,15-H), 6.65 (1H, s, 10-H); 8.20 (3H, br s, NH). λ_{max} 372 (ε 59,900) and 654 nm (17,900). *m/e* (%), 556 (100), 255 (23), 227 (21).

2,7,13,18-*Tetraethyl*-3,8,12,17-*tetramethyl*-1,19-*bilindione* ("*Etiobiliverdin IVα*") (**44**)

This compound was prepared, as described for **42**, from **43** (150 mg; 0.02 mMole), and was obtained from CH_2Cl_2/hexane as dark blue needles (73 mg; 73%) with mp. 270–272°. (Found: C, 74.59; H, 7.74; N, 11.16. $C_{31}H_{38}N_4O_2$ requires: C, 74.67; H, 7.68; N, 11.24%). NMR, δ, 1.06, 1.11 (each 6H, t, CH_3CH_2); 2.08, 2.16 (each 6H, s, Me); 2.27, 2.46 (each 4H, q, CH_2CH_3); 5.85 (2H, s, 5,15-H); 6.62 (1H, s, 10-H); 8.60 (3H, br s, NH). λ_{max} 366 (ε 50,500) and 654 nm (14,700). *m/e* (%), 498 (100), 249 (42), 227 (27).

Acknowledgements This research was generously supported by grants from the National Science Foundation and the National Institutes of Health (HL 22252).

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