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BILE PIGMENT STUDIES—VII[1]

NEW SYNTHESES OF BILIVERDIN-IXα DIMETHYL ESTER AND TWO RELATED MONO-VINYL–MONO-ETHYL ISOMERS

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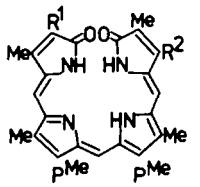
Abstract—Using the recently developed route through di-t-butyl b-bilene-1,19-dicarboxylates, new syntheses of biliverdin-IXα dimethyl ester (**1**) and two related mono-vinyl–mono-ethyl isomers (**2**) and (**3**), are described. The two dihydro-derivatives of (**1**) are important in connection with biosynthetic studies of the origin of algal biliproteins.

We recently reported an efficient new method for the rapid and efficient synthesis of biliverdins (1,19-bilindiones).[1,2] The route was shown to produce yields of biliverdins well in excess of those in Ref. 3 and was advantageous from several points of view. Most important was the fact that the approach utilized standard pyrrole procedures which have been particularly optimized over the past few years; furthermore, the key lactam rings at the termini of pigments were introduced at the tetrapyrrole stage by way of an extremely efficient transformation of a pyrrolic *t*-butyl ester using bromine in trifluoroacetic acid. Earlier syntheses of biologically important bile pigments usually required attachment of a previously synthesized pyrrolinone to a pyrrole to form a pyrromethanone or pyrromethenone intermediate, and such diversely substituted pyrrolinones are not nearly as accessible as the correspondingly substituted pyrrole t-butyl ester comprising the equivalent building block. For the specific cases of unsymmetrically substituted biliverdins, it was shown that an approach via di-t-butyl b-bilene-1,19-dicarboxylates was the most efficient, and this was demonstrated in the synthesis of two model compounds.

In the present paper, we describe new syntheses of biliverdins of known, and possible, biological importance. Biliverdin-IXα dimethyl ester (**1**) was the first objective of our expansion of the route to afford bile pigments of natural importance, and then the two mono-vinyl–mono-ethyl isomers (**2**) and (**3**) were synthesized. These last two compounds, which will eventually be required in radioactive form, are of importance with regard to the latter steps (after heme) in the biosynthesis of algal biliproteins such as phycocyanin.[4,5] A rapid, efficient synthesis of these compounds, which would be amenable to insertion of an isotopic label, was therefore required.

Synthesis of biliverdin-IXα dimethyl ester (**1**)

On account of earlier successes,[3] the 2-chloroethyl group was chosen as a protected vinyl substituent. The required b-bilene-1,19-dicarboxylic acid (**4**) was approached from pyrromethanes as follows. The 5-acetoxymethylpyrrole (**5**) and 5-unsubstituted pyrrole (**6**) were condensed in acetic acid containing a catalytic quantity of toluene p-sulfonic acid[6] to give



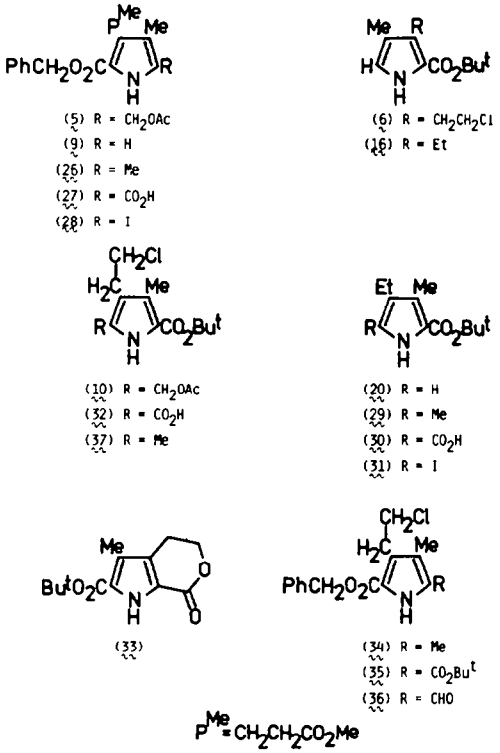
(**1**) R^1= R^2= $CH{=}CH_2$
(**2**) R^1= Et; R^2= $CH{=}CH_2$
(**3**) R^1= $CH{=}CH_2$; R^2= Et
(**14**) R^1= R^2= CH_2CH_2Cl
(**19**) R^1= Et; R^2= CH_2CH_2Cl
(**25**) R^1= CH_2CH_2Cl; R^2= Et

an 81% yield of the pyrromethane-5,5′-dicarboxylate (**7**); catalytic hydrogenolysis gave the 5′-carboxylic acid (**8**) in near quantitative yield. Meanwhile, similar condensation of the 5-unsubstituted pyrrole (**9**) and the 5-acetoxymethylpyrrole (**10**) gave a 77% yield of the isomeric pyrromethane (**11**), which was debenzylated [to give (**12**)], and then formylated, using the Vilsmeier procedure, to give the 5′-formylpyrromethane (**13**) in 64% yield from (**11**). A simple modification of the Vilsmeier formylation step, using 1-^{14}C-dimethylformamide, would enable the corresponding labelled pyrromethane (and eventually, labelled biliverdin) to be prepared.

Condensation of the pyrromethanes (**8**) and (**13**) in methanol in the presence of toluene p-sulfonic acid, gave an 85% yield of the b-bilene-1,19-dicarboxylic acid (**4**) after exchange of the anion using hydrogen bromide gas and concomitant removal of the t-butyl esters; this was treated with bromine in trifluoroacetic acid under strictly anaerobic conditions, and gave a 42% yield of the bis-(2-chloroethyl)-biliverdin dimethyl ester (**14**). With potassium hydroxide in pyridine/methanol, a 54% yield of biliverdin-IXα dimethyl ester (**1**) was obtained. This was identical, in all respects, with an authentic sample.

Synthesis of the mono-ethyl–mono-vinyl derivatives (**2**) and (**3**)

For the synthesis of (**2**), the pyrromethane (**15**) was prepared in 77% yield from pyrroles (**5**) and (**16**) and this was hydrogenated to give a 90% yield of the



5′-carboxylic acid (**17**). Pyrromethane (**13**) was then condensed with this, as previously, and gave an 87% yield of the b-bilene (**18**); with bromine in trifluoroacetic acid, (**18**) gave directly a 40% yield of the mono-(2-chloroethyl)-biliverdin dimethyl ester (**19**). With potassium hydroxide in pyridine/methanol, a 76% yield of the required mono-vinyl-biliverdin (**2**) was obtained.

Biliverdin (**3**) was likewise approached by condensation of pyrroles (**5**) and (**20**) to give (**21**; 77.5%), which was hydrogenolyzed (to give **22**) and then formylated, once more by the Vilsmeier procedure, to give (**23**; 66%). Treatment of (**23**) with the pyrromethane (**8**) afforded an 83% yield of the b-bilene (**24**) which was smoothly converted into the biliverdin dimethyl ester (**25**; 38%) by treatment with bromine in trifluoroacetic acid under nitrogen. Finally, dehydrochlorination (potassium hydroxide/pyridine/methanol) gave a 69% yield of the biliverdin dimethyl ester (**3**).

Synthetic procedures with monopyrroles

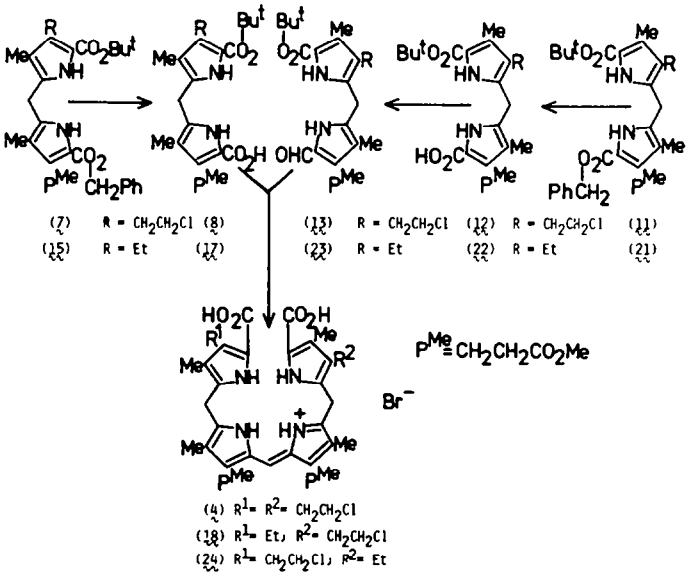
The monopyrroles required for the pyrromethane syntheses were prepared using standard methodology. Thus, pyrrole (**26**)[7] was treated with lead tetra-acetate and gave a 95% yield of the acetoxymethylpyrrole (**5**). The same methylpyrrole (**26**) was trichlorinated with sulfuryl chloride and, after hydrolysis, gave a 73% yield of the pyrrole carboxylic acid (**27**) which was iodinated (to give **28**) and then treated with zinc dust in acetic acid to give a 90% yield of the unsubstituted pyrrole (**9**).

In a similar series of reactions, the known pyrrole (**29**)[8] was trichlorinated and hydrolyzed to give (**30**) (75% yield), then iodinated to give a 78% yield of (**31**) and hydrogenated over Adams catalyst to give (**20**) in 99% yield. An attempt to iodinate the chloroethylpyrrole carboxylic acid (**32**) gave a 70% yield of the corresponding pyrrole lactone (**33**) produced, no doubt, by nucleophilic attack of the intermediate carboxylate anion on the chloroethyl side chain.

Trichlorination of the chloroethylpyrrole (**34**)[9] gave the pyrrole mixed ester (**35**) (60% yield) after treatment with t-butyl alcohol,[10] along with the formylpyrrole (**36**), a by-product of incomplete chlorination of the methyl in the first step. Pyrrole (**10**) was obtained from 2-methylpyrrole (**37**) by treatment with lead tetra-acetate.

EXPERIMENTAL

Mps were measured on a hot-stage apparatus, and are uncorrected. Silicagel 60 (70-230 mesh; Merck) or neutral alumina (Merck) was used for column chromatography, and preparative TLC was carried out on 20 × 20 cm glass plates coated with Merck GF 254 silica gel (1 mm thick). Analytical TLC was performed using Merck silica gel 60 F 254 precoated sheets (0.2 mm). Electronic absorption spectra were measured on a Hewlett–Packard 8450A spectrophotometer (solns in dichloromethane) and proton



NMR spectra were measured either at 90 MHz (Varian EM-390) or at 360 MHz (Nicolet NT-360) in deuteriochloroform soln. Elemental analyses were performed at the Berkeley Microanalytical Laboratory, Department of Chemistry, UC Berkeley.

2-Benzyloxycarbonyl-3-(2-methoxycarbonylethyl)-4-methylpyrrole-5-carboxylic acid (**27**).

Benzyl 3-(2-methoxycarbonylethyl)-4,5-dimethylpyrrole (**26**)[7] (10 g) in dry carbon tetrachloride (300 ml) was treated, with stirring, with sulfuryl chloride (8 ml) in carbon tetrachloride (50 ml) during 30 min. (The monochloro-derivative precipitated initially, but soon went back into solution). The mixture was stirred for 3 h at room temperature while being monitored by NMR spectroscopy. Evaporation under vacuum gave an oily product which was taken up in dioxan (400 ml) and treated with a solution of sodium acetate (52 g) in water (300 ml); this mixture was heated under reflux for 2 h and then stirred overnight at room temp. The resulting mixture was extracted with ether (3 × 500 ml) and this was extracted with aqueous sodium bicarbonate (2 × 100 ml) and then with aqueous sodium carbonate (2 × 100 ml). The aqueous phases were combined and air was rapidly passed through the solution for 30 min (to remove dioxan and ether). Sulphur dioxide gas was then bubbled into the mixture until pH 6 was reached, whereupon the pyrrole precipitated. The product was collected by filtration, washed with water, and then dried to give 8.0 g (73%), m.p. 203–205°. Found: C, 62.32; H, 5.49; N, 3.98. $C_{18}H_{19}NO_6$ requires: C, 62.60; H, 5.55; N, 4.06%. δ, ppm, 2.20 (s, 3H, Me), 2.3–3.0 (m, 4H, CH_2CH_2CO), 3.55 (s, 3H, OMe), 5.28, 7.42 (each s, 2H, 5H, CH_2Ph).

Benzyl 5 - Iodo - 3 - (2 - methoxycarbonylethyl) - 4 - methylpyrrole - 2 - carboxylate (**28**)

The foregoing pyrrole (**27**) (4 g) in methanol (30 ml) and water (30 ml) containing sodium bicarbonate (2.8 g) was stirred at 60° and treated dropwise with a solution of iodine (2.84 g) and potassium iodide (4.5 g) in methanol (40 ml) and water (10 ml). After complete addition at a rate which did not allow the mixture to become significantly darkened, water (40 ml) was added and the mixture was stirred for a further 1 h at the same temp. The mixture was cooled, the white solid was filtered off, dried (vacuum oven) and then recrystallized from dichloromethane/hexane to give 3.96 g (80%) of the iodopyrrole, m.p. 117°. Found: C, 47.76; H, 4.21; N, 3.22. $C_{17}H_{18}INO_4$ requires: C, 47.77; H, 4.21; N, 3.27%. δ, ppm, 1.95 (s, 3H, Me), 2.48, 3.10 (each t, 2H, CH_2CH_2CO), 3.60 (s, 3H, OMe), 5.28, 7.30 (each s, 2H, 5H, CH_2Ph), 9.00 (br s, 1H, NH).

Benzyl 3 - (2 - Methoxycarbonylethyl) - 4 - methylpyrrole - 2 - carboxylate (**9**)

The foregoing iodopyrrole (2.4 g) was added to a suspension of zinc dust (3 g) in acetic acid (150 ml) and the mixture was heated under reflux for 3 h. Additional zinc dust (3 g) was added after 1 h. The mixture was then cooled, treated with conc. hydrochloric acid (0.2 ml) and the zinc was filtered off. After dilution of the filtrate with ice cold water (250 ml) the precipitate was collected, dried (vacuum oven) and then recrystallized from dichloromethane/hexane to give 1.52 g (90%) of the pyrrole, m.p. 60°. Found: C, 67.57; H, 6.35; N, 4.65. $C_{17}H_{19}NO_4$ requires: C, 67.76; H, 6.36; N, 4.65%. δ, ppm, 2.05 (s, 3H, Me), 2.52, 3.05 (each t, 2H, CH_2CH_2CO), 3.68 (s, 3H, OMe), 5.30 (s, 2H, CH_2Ph), 6.65 (d J = 3 Hz, 1H, 5-H), 7.40 (s, 5H, Ph), 9.00 (br s, 1H, NH).

Benzyl 5 - acetoxymethyl - 3 - (2 - methoxycarbonylethyl) - 4 - methylpyrrole - 2 - carboxylate (**5**)

Benzyl 3 - (2 - methoxycarbonylethyl) - 4,5 - dimethylpyrrole - 2 - carboxylate (**26**)[7] (5.0 g) in acetic acid (60 ml) and acetic anhydride (1.5 ml) was stirred and treated over 1 h with portions of lead tetra - acetate (7.5 g, total) at room temperature. After stirring overnight the mixture was added dropwise to water (250 ml) with vigorous stirring. The product was filtered off and recrystallized from dichloromethane/hexane to give the acetoxymethylpyrrole (5.7 g, 95%) as white crystals, m.p. 97°. Found: C, 64.26; H, 6.19; N, 3.75. $C_{20}H_{23}NO_6$ requires: C, 64.33; 6.21; N, 3.75%. δ, ppm, 2.10 (s, 3H, Me), 2.32, 3.18 (each t, 2H, CH_2CH_2CO), 3.65 (s, 3H, OMe), 5.00 (s, 2H, CH_2O), 5.30, 7.38 (each s, 2H, 5H, CH_2Ph), 9.00 (br s, 1H, NH).

t - Butyl 5 - acetoxymethyl - 4 - (2 - chloroethyl) - 3 - methylpyrrole - 2 - carboxylate (**10**)

This pyrrole was likewise prepared from t - butyl 4 - (2 - chloroethyl) - 3,5 - dimethylpyrrole - 2 - carboxylate (**37**)[11] (8 g) and lead tetra - acetate (15 g) in acetic acid (300 ml) and acetic anhydride (14 ml). The product (8 g; 82%) was recrystallized from dichloromethane/hexane, and had m.p. 126°. Found: C, 57.05; H, 6.98; N, 4.29. $C_{15}H_{22}NO_4$ requires: C, 57.05; H, 6.97; N, 4.43%. δ, ppm, 1.52 (s, 9H, t - Bu), 2.05, 2.20 (s, 3H, Me), 2.86, 3.50 (each t, 2H, CH_2CH_2Cl), 5.00 (s, 2H, CH_2), 8.92 (br s, 1H, NH).

2 - t - Butoxycarbonyl - 4 - ethyl - 3 - methylpyrrole - 5 - carboxylic acid (**30**)

t - Butyl 4 - ethyl - 3,5 - dimethylpyrrole - 2 - carboxylate (**29**)[8] in carbon tetrachloride (200 ml) was treated with sulfuryl chloride as described in the synthesis of pyrrole (**28**). A 75% yield (2.5 g) of the pyrrole carboxylic acid was obtained, m.p. 198 - 203°. Found: C, 61.85; H, 7.43; N, 5.32. $C_{13}H_{19}NO_4$ requires: C, 61.64; H, 7.56; N, 5.53%. δ, ppm, 1.10 (t, 3H, CH_2CH_3), 1.58 (s, 9H, t - Bu), 2.25 (s, 3H, Me), 2.80 (q, 2H, CH_2CH_3), 9.40 (br s, 1H, NH).

t - Butyl 4 - ethyl - 5 - iodo - 3 - methylpyrrole - 2 - carboxylate (**31**)

The foregoing pyrrole carboxylic acid (6 g) was treated with iodine (5.8 g) and potassium iodide (9.2 g) in methanol (80 ml) as described above in the synthesis of pyrrole (**28**). and gave 6.2 g (78.1%) of the product, m.p. 119–121°. Found: C, 42.89; H, 5.40; N, 4.10. $C_{12}H_{18}INO_2$ requires: C, 42.95; H, 5.41; N, 4.11%. δ, ppm, 1.00 (t, 3H, CH_2CH_3), 1.58 (s, 9H, t - Bu), 2.25 (s, 3H, Me), 2.40 (q, 2H, CH_2CH_3), 8.90 (br s, 1H, NH).

When an attempt was made to perform the same iodination reaction using 2 - t - butyloxycarbonyl - 4 - (2 - chloroethyl) - 3 - methylpyrrole - 5 - carboxylic acid (**32**) (3 g) the corresponding pyrrole lactone (**33**) was obtained (1.82 g; 70%), mp 116–117°. Found: C, 62.33; H, 6.96; N, 5.71. $C_{13}H_{17}NO_4$ requires: C, 62.14; H, 6.82; N, 5.57%. δ, ppm, 1.58 (s, 9H, t - Bu), 2.20 (s, 3H, Me), 2.82, 4.60 (each t, 2H, CH_2CH_2CO), 9.48 (br s, 1H, NH).

t - Butyl 4 - ethyl - 3 - methylpyrrole - 2 - carboxylate (**20**)

The iodopyrrole (**31**) (6.0 g) was dissolved in methanol (150 ml) containing sodium acetate (7.0 g) and Adams catalyst (70 mg) and hydrogenated at room temp. and atmospheric pressure until uptake of hydrogen ceased. The catalyst was filtered off through Celite and the solvent was evaporated to give a residue which was taken up in dichloromethane (100 ml) and washed with water (100 ml), then dried (Na_2SO_4), and evaporated to dryness. The residue was crystallized from dichloromethane/hexane and gave 3.7 g (99%) of the product, m.p. 95°. Found: C, 68.76; H, 9.08; N, 6.64. $C_{12}H_{19}NO_2$ requires: C, 68.87; H, 9.15; N, 6.69%. δ, ppm, 1.18 (t, 3H, CH_2CH_3), 1.58 (s, 9H, t - Bu), 2.28 (s, 3H, Me), 2.46 (q, 2H, CH_2CH_3), 6.56 (d J = 3 Hz, 1H, 5–H), 9.16 (br s, 1H, NH).

Benzyl 5 - t - butoxycarbonyl - 3 - (2 - chloroethyl) - 4 - methylpyrrole - 2 - carboxylate (**35**)

Benzyl 3 - (2 - chloroethyl) - 4,5 - dimethylpyrrole - 2 - carboxylate (**34**)[9] (4.2 g) was trichlorinated using sulfuryl chloride (4.5 ml) in carbon tetrachloride (250 ml) as solvent, as described above for compound (**27**). The oily product was treated with dry t-butyl alcohol (100 ml) and anhydrous sodium acetate (6.2 g) and stirred for 24 h. The mixture was

then diluted with dichloromethane (200 ml), washed with water (2 × 100 ml) and the organic phase was dried (Na_2SO_4) and evaporated to give a residue which was purified by chromatography on a silica gel column (elution with 20% ethyl acetate in cyclohexane). The first fraction afforded, after evaporation, the desired product which was recrystallized from dichloromethane/hexane to give 3.25 g (60%), m.p. 60–61°. Found: C, 63.61; H, 6.33; N, 3.67. $C_{20}H_{24}ClNO_4$ requires: C, 63.57; H, 6.35; N, 3.70%. δ, ppm, 1.60 (s, 9H, t - Bu), 2.30 (s, 3H, Me), 3.18, 3.60 (each t, 2H, CH_2CH_2), 5.32, 7.40 (each s, 2H, 5H, CH_2Ph), 9.52 (br s, 1H, NH). Further elution of the column gave eluates from which benzyl 3 - (2 - chloroethyl) - 5 - formyl - 4 - methylpyrrole - 2 - carboxylate (**36**) (800 mg), m.p. 115–116° was isolated. Found: C, 62.69; H, 5.33; N, 4.49. $C_{16}H_{16}ClNO_3$ requires: C, 62.84; H, 5.23; N, 4.58%. δ, ppm, 2.35 (s, 3H, Me), 3.15, 3.65 (each t, 2H, CH_2CH_2), 5.32, 7.42 (each s, 2H, 5H, CH_2Ph), 9.80 (br s, 1H, NH), 9.88 (s, 1H, CHO).

2 - t - Butoxycarbonyl - 4 - (2 - chloroethyl) - 3 - methylpyrrole - 5 - carboxylic acid (**32**)

The foregoing pyrrole mixed ester (**35**) (5 g) in tetrahydrofuran (100 ml) and triethylamine (0.1 ml) containing 10% palladised charcoal (550 mg) was hydrogenated at room temp and atmospheric pressure until uptake of hydrogen ceased. The mixture was filtered through Celite and the filtrate was evaporated to give a residue which was crystallized from tetrahydrofuran/hexane to give the pyrrole carboxylic acid (3.54 g; 90%) as a white powder, m.p. > 200° (dec). Found: C, 54.36; H, 6.16; N, 4.92. $C_{13}H_{18}ClNO_4$ requires: C, 54.26; H, 6.26; N, 4.86%. δ, ppm, 1.58 (s, 9H, t - Bu), 2.30 (s, 3H, Me), 3.20, 3.68 (each t, 2H, CH_2CH_2), 9.80 (br s, 1H, NH).

Benzyl 5' - t - butoxycarbonyl - 3' - (2 - chloroethyl) - 4 - (2-methoxycarbonylethyl) - 3,4' - dimethylpyrromethane - 5 - carboxylate (**11**)

Benzyl 3 - (2 - methoxycarbonylethyl) - 4 - methylpyrrole-2 - carboxylate (**9**) (839 mg) in acetic acid (50 ml) was treated with t - butyl 5 - acetoxymethyl - 4 - (2 - chloroethyl) - 3-methylpyrrole - 2 - carboxylate (**10**) (879 mg) along with p-toluene sulfonic acid hydrate (27 mg) before being stirred under nitrogen at 45° for 4 h. The mixture was poured into water (100 ml), extracted with dichloromethane (100 ml) and this was washed with aqueous sodium bicarbonate, then water, and dried (Na_2SO_4), before being evaporated to dryness. The crude product was chromatographed on a silica column (elution with 20% ethyl acetate/cyclohexane) and the appropriate eluates gave an oil (1.20 g, 77%) which could not be induced to crystallize. δ, ppm, 1.60 (s, 9H, t - Bu), 2.00, 2.28 (each s, 3H, Me), 2.40–3.50 (each t, 8H, CH_2CH_2Cl, CH_2CH_2CO), 3.69 (s, 3H, OMe), 3.85 (s, 2H, CH_2), 5.30, 7.30 (each s, 2H, 5H, CH_2Ph), 8.70, 8.90 (each br s, 1H, NH).

5' - t - Butoxycarbonyl - 3' - (2 - chloroethyl) - 4 - (2 - methoxycarbonylethyl) - 3,4' - dimethylpyrromethane - 5 - carboxylic acid (**12**)

The foregoing pyrromethane (**11**) (1.5 g) in tetrahydrofuran (50 ml) containing triethylamine (0.1 ml) and 10% palladised charcoal (150 mg) was hydrogenated at room temperature and atmospheric pressure until uptake of hydrogen ceased. The catalyst was removed by filtration through Celite and the filtrate was evaporated to give a residue which was precipitated from tetrahydrofuran/hexane, to give the product (1.23 g; 98%), as an amorphous solid. δ, ppm, 1.52 (s, 9H, t - Bu), 2.10, 2.20 (each s, 3H, Me), 2.48–3.58 (m, 8H, CH_2CH_2Cl, CH_2CH_2CO), 3.68 (s, 3H, OMe), 3.88 (s, 2H, CH_2).

t - Butyl 3' - (2 - chloroethyl) - 5 - formyl - 4 - (2 - methoxycarbonylethyl)-3,4' - dimethylpyrromethane - 5' - carboxylate (**13**)

The foregoing pyrromethane carboxylic acid (**12**) (980 mg) in dry dichloromethane (40 ml) was stirred with toluene p-sulfonic acid hydrate (980 mg) in dry methanol (20 ml) for 40 min at room temp. The mixture was diluted with dichloromethane (100 ml) and was washed with 2% aqueous sodium carbonate, then water, dried (Na_2SO_4), and evaporated to dryness. The resulting pale yellow oil was dissolved in dry dichloromethane (15 ml) and added dropwise to a suspension (at 0°) of calcium carbonate (2 g) in dichloromethane (5 ml) containing Vilsmeier complex [obtained from phosphoryl chloride (1.9 ml) and dimethylformamide (1.5 ml)]; after complete addition the mixture was stirred at room temperature for 1.5 h and then 15% aqeous sodium acetate was added (to pH 7) before the mixture was stirred vigorously overnight. Then aqueous sodium carbonate was added (to pH 8) and after standing for 10 min the mixture was filtered to remove calcium carbonate and the organic phase was evaporated, washed with water (2 × 50 ml), and then dried (Na_2SO_4) and evaporated to dryness. After column chromatography on silica gel (elution with 30% ethyl acetate in cyclohexane) the required eluates were evaporated to give the formylpyrromethane, 602 mg (64%), m.p. 148–149°. Found: C, 61.34; H, 6.91; N, 6.14. $C_{23}H_{31}ClN_2O_5$ requires: C, 61.26; H, 6.88; N, 6.21%. δ, ppm, 1.50 (s, 9H, t - Bu), 2.00, 2.20 (each s, 3H, Me), 2.30–3.50 (m, 8H, CH_2CH_2Cl, CH_2CH_2CO), 3.62 (s, 3H, OMe), 3.95 (s, 2H, CH_2), 9.55 (s, 1H, CHO), 9.75 (br s, 2H, NH).

Benzyl 5' - t - butoxycarbonyl - 4' - ethyl - 4 - (2 - methoxycarbonylethyl) - 3,3' - dimethypyrromethane - 5 - carboxylate (**15**)

Benzyl 5 - acetoxymethyl - 3 - (2 - methoxycarbonylethyl)-4 - methylpyrrole - 2 - carboxylate (**5**) (1.12 g) and t-butyl 3 - ethyl - 4 - methylpyrrole - 2 - carboxylate (**16**)[12] (627 mg) and toluene p-sulfonic acid hydrate (27 mg) were treated as mentioned above for synthesis of compound (**11**), and after the usual workup afforded the pyrromethane (1.20 g; 77%), m.p. 115–116°, after crystallization from dichloromethane/hexane. Found: C, 69.06; H, 7.35; N, 5.47. $C_{30}H_{38}N_2O_6$ requires: C, 68.94; H, 7.33; N, 5.36%. δ, ppm, 1.10 (t, 3H, CH_2CH_3), 1.58)s, 9H, t - Bu), 1.82, 1.88 (each s, 3H, Me), 2.10–3.32(m, 6H, CH_2CH_2CO, CH_2CH_3), 3.52 (s, 3H, OMe), 3.75 (s, 2H, CH_2), 5.20, 7.28 (each s, 2H, 5H, CH_2Ph), 8.62, 8.98 (each br s, 1H, NH).

5' - t - Butoxycarbonyl - 4' - ethyl - 4 - (2 - methoxycarbonylethyl) - 3,3' - dimethylpyrro - methane - 5 - carboxylic acid (**17**)

The foregoing pyrromethane (**15**) (1.0 g) in tetrahydrofuran (50 ml) and triethylamine (0.1 ml) containing 10% palladised charcoal was hydrogenated at room temp. and atmospheric pressure as described in the synthesis of compound (**12**). After the usual workup the pyrromethane acid was obtained in 90% yield (744 mg), as an amorphous powder. δ, ppm, 1.12 (t, 3H, CH_2CH_3), (s, 9H, t - Bu), 1.80-1.88 (each s, 3H, Me), 2.05–3.30 (m, 6H, CH_2CH_2CO, CH_2CH_3), 3.52 (s, 3H, OMe), 3.75 (s, 2H, CH_2), 8.65, 9.00 (each br s, 1H, NH).

Benzyl 5' - t - butoxycarbonyl - 4' - (2 - chloroethyl) - 4 - (2-(2 - methoxycarbonylethyl) - 3,3' - dimethylpyrromethane - 5-carboxylate (**7**)

Acetoxymethylpyrrole (**5**) (1.5 g) and unsubstituted pyrrole (**6**) (970 mg) were treated with toluene p-sulfonic acid hydrate (35 mg) in acetic acid (50 ml) as described in the synthesis of pyrromethane (**11**) and gave an 81% yield (1.8 g) of pyrromethane, m.p. 110°, from dichloromethane/hexane.

Found: C, 64.73; H, 6.70; N, 5.08. $C_{30}H_{37}ClN_2O_6$ requires: C, 64.69; H, 6.64; N, 5.03%. δ, ppm, 1.52 (s, 9H, t - Bu), 1.95 (s, 6H, Me), 2.1–3.4 (m, 8H, CH_2CH_2Cl, CH_2CH_2CO), 3.58 (s, 3H, OMe), 3.75 (s, 2H, CH_2), 5.22, 7.30 (each s, 2H, 5H, CH_2Ph), 8.85, 9.10 each br s, 1H, NH).

5′ - t - Butoxycarbonyl - 4′ - (2 - chloroethyl) - 3′ - ethyl - 4 - (2 - methoxycarbonylethyl) - 5 - carboxylic acid (**8**)

The foregoing pyrromethane (**7**) (800 mg) in tetrahydrofuran (50 ml) and triethylamine was hydrogenated over 10% palladized charcoal as described in the synthesis of compound (**12**). The product could not be induced to crystallize, was precipitated as an amorphous powder (616 mg; 92%), and was used immediately in b - bilene syntheses.

Benzyl 5′ - t - butoxycarbonyl - 3′ - ethyl - 4 - (2 - methoxycarbonylethyl) - 3,4′ - dimethylpyrromethane - 5 - carboxylate (**21**).

Benzyl 5 - acetoxymethyl - 3 - (2 - methoxycarbonylethyl)-4 - methylpyrrole - 2 - carboxylate (**5**) (1.12 g), t-butyl 3-(2 - chloroethyl) - 4 - methylpyrrole - 2 - carboxylate (**20**)[6] (970 mg) and toluene p-sulfonic acid hydrate (35 mg) in acetic acid (50 ml) were treated as described for the synthesis of pyrromethane (**11**) and gave a 77.5% yield (1.21 g) of an oily pyrromethane. δ, ppm, 0.95 (t, 3H, CH_2CH_3), 1.50 (s, 9H, t - Bu), 1.90, 2.20 (each s, 3H, Me), 2.30–3.30 (m, 6H, CH_2CH_2CO, CH_2CH_3), 3.75 (s, 2H, CH_2), 5.25, 7.30 (each s, 2H, 5H, CH_2Ph), 8.50, 8.80 (each br s, 1H, NH).

t - Butyl 3′ - ethyl - 5 - formyl - 4 - (2 - methoxycarbonylethyl - 3,4′ - dimethylpyrromethane - 5′ - carboxylate (**23**)

The foregoing pyrromethane diester (**21**) (1.20 g) in tetrahydrofuran (50 ml) containing triethylamine (0.1 ml) and 10% palladised charcoal (150 mg) was hydrogenated as described in the synthesis of compound (**12**). After the usual work up, the resulting pyrromethane carboxylic acid (**22**) was dissolved in dichloromethane (40 ml) and treated with toluene p-sulfonic acid hydrate (1.68 g) and Vilsmeier complex [phosphoryl chloride (1.9 ml) and dimethylformamide (1.5 ml)] as described above in the synthesis of pyrromethane (**13**). After chromatography on silica gel (elution with 30% ethyl acetate in cyclohexane) the product was obtained, m.p. 111–112°, in 66% yield (765 mg). Found: C, 66.51; H, 7.78; N, 6.77. $C_{23}H_{32}N_2O_3$ requires: C, 66.32; H, 7.74; N, 6.73%. δ, ppm, 0.98 (t, 3H, CH_2CH_3), 1.52 (s, 9H, t - Bu), 2.00, 2.20 (each s, 3H, Me), 2.20–2.62 (m, 4H, CH_2CH_2CO), 2.95 (q, 2H, CH_2CH_3), 3.62 (s, 3H, OMe), 3.88 (s, 2H, CH_2), 9.52 (s, 1H, CHO), 9.60 (br s, 2H, NH).

3 - Ethyl - 18 - (2 - chloroethyl) - 8,12 - bis - (2 - methoxycarbonylethyl) - 2,7,13,17 - tetramethyl - b - bilene-1,19 - dicarboxylic acid hydrobromide (**24**)

The pyrromethane carboxylic acid (**8**) (234 mg) and the formylpyrromethane (**23**) (208 mg) in dichloromethane (10 ml) were treated with toluene p-sulfonic acid hydrate (475 mg) in methanol (20 ml) under nitrogen during 16 h. The mixture was washed with 2% aqueous sodium carbonate (50 ml) and the organic phase was dried (Na_2SO_4) and evaporated to dryness. Dichloromethane (20 ml) was then added and hydrogen bromide gas was passed through the solution for 5 sec (color: yellow to red) before the solvent was rapidly evaporated; dry benzene (20 ml) was added and this was likewise removed rapidly under vacuum. Upon trituration with ether the red b - bilene crystallized and was filtered off to give 327 mg (83%), m.p. > 300°. Found: C, 56.36; H, 6.02; N, 6.92. $C_{37}H_{45}BrClN_4O_8$ requires: C, 56.30; H, 5.74; N, 7.09%. δ, ppm, 1.06 (t, 3H, CH_3CH_2), 2.03, 2.07, 2.12, 2.27 (each s, 3H, Me), 2.54 - 3.25 (m, 14H, CH_2CH_2Cl, CH_2CH_2CO, CH_2CH_3), 3.61 (s, 6H, OMe), 4.34 (s, 4H, 5,15 - CH_2), 7.62 (s, 1H, - CH=), 10.64, 10.88, 13.26, 13.29 (each s, 1H, NH). λ_{max} 504 nm (ϵ 45,500).

2.Ethyl - 17 - (2 - chloroethyl) - 8,12 - bis - (2 - methoxycarbonylethyl) - 3,7,13,18 - tetramethyl - b - bilene - 1,19 - dicarboxylic acid hydrobromide (**18**)

This b - bilene was likewise prepared from the pyrromethane acid (**17**) (372 mg), formylpyrromethane (**13**) (388 mg), and toluene p-sulfonic acid hydrate (818 mg) and gave 592 mg (87%) of the b - bilene, m.p. > 300°. Found: C, 55.22; H, 5.90; N, 6.59. $C_{37}H_{45}BrClN_4O_8$ requires: C, 55.60; H, 5.76; N, 7.01%. δ, ppm, 1.04 (t, 3H, CH_2CH_3), 2.16, 2.17, 2.18, 2.25 (each s, 3H, Me), 2.51–3.50 (m, 14H, CH_2CH_3, CH_2CH_2Cl, CH_2CH_2CO), 3.61 (s, 6H, OMe), 4.37, 4.39 (each s, 2H, 5,15 - CH_2), 7.62 (s, 1H, –CH=), 10.63, 10.86, 13.29, 13.30 (each s, 1H, NH). λ_{max} 504 nm (ϵ 45,750).

2,17 - Bis - (2 - chloroethyl) - 8,12 - bis - (2 - methoxycarbonylethyl) - 3,7,13,18 - tetramethyl - b - bilene - 1,19-dicarboxylic acid hydrobromide (**4**)

This b - bilene was similarly prepared from the pyrromethane carboxylic acid (**8**) (108 mg), formylpyrromethane (**13**) (105 mg), and toluene p-sulfonic acid hydrate (235 mg), and the product was obtained in 85% yield (163 mg), m.p. > 300°. Found: C, 52.66; H, 5.62; N, 6.66. $C_{37}H_{44}Cl_2N_4O_8 \cdot H_2O$ requires: C, 52.79; H, 5.46; N, 6.65%. δ, ppm, 2.10 (s, 6H, Me), 2.15, 2.25 (each s, 3H, Me), 2.5–3.5 (m, 16H, CH_2CH_2Cl, CH_2CH_2CO), 3.65 (s, 6H, OMe), 4.40, 4.42 (each s, 2H, CH_2), 7.60 (s, 1H, –CH=), 10.85, 12.55, 12.60 (each br s, 2H, 1H, 1H, NH). λ_{max} 504 nm (ϵ 39,600).

2 - Ethyl - 17 - (2 - chloroethyl) - 21,24 - dihydro - 8,12 - bis-(2 - methoxycarbonylethyl) - 3,7,13,18 - tetramethylbilin - 1,19 - dione (**19**)

Trifluoroacetic acid (70 ml) was degassed by bubbling nitrogen through it for 30 min, and then the b - bilene hydrobromide (**18**) (350 mg) was introduced and the mixture was stirred under nitrogen for 15 min at room temperature. The solution was cooled to $-5°$ before addition of bromine (0.1 ml). After stirring for a further 1.5 h at $-5°$ (color: red to blue), the mixture was poured onto sodium bicarbonate (50 g) and dichloromethane (50 ml) was added. Water (50 ml) was added and the organic layer was separated, dried (Na_2SO_4), and evaporated to give a blue residue which was chromatographed on silicagel thick layer plates (elution with 5% methanol in dichloromethane). The blue product was recovered from the silica gel and evaporation gave a solid which was crystallized from dichloromethane/hexane to give the biliverdin, (115 mg; 40%), m.p. 225–227°. Found: C, 64.63; H, 6.31; N, 8.12. $C_{35}H_{41}ClN_4O_6$ requires: C, 64.76; H, 6.32; N, 8.63%. δ, ppm, 1.04 (t, 3H, CH_2CH_3), 1.86, 2.08 (each s, 3H, Me), 2.09 (s, 6H, Me), 2.26–3.00 (m, 14H, CH_2CH_2Cl, CH_2CH_2CO, CH_2CH_3), 3.67 (s, 6H, OMe), 5.89, 5.92, 6.75 (each s, 1H, –CH=), 8.2 (br s, 3H, NH). λ_{max} 373 nm (ϵ 49,500), 6363 (14,500).

2 - (2 - Chloroethyl) - 17 - ethyl - 21,24 - dihydro - 8,12 - bis-(2 - methoxycarbonylethyl) - 3,7,13,18 - tetramethylbilin - 1,19 - dione (**25**)

This bilindione was likewise prepared from the b - bilene hydrobromide (**24**) (350 mg) and gave 109 mg (38%) of the biliverdin, m.p. 226–228°. Found: C, 63.24; H, 6.09; N, 8.06. $C_{35}H_{41}ClN_4O_6 \cdot \frac{1}{2}H_2O$ requires: C, 63.87; H, 6.38; N, 8.51%. δ, ppm, 1.18 (t, 3H, CH_2CH_3), 1.81, 2.14 (each s, 3H, Me), 2.07 (s, 6H, Me), 2.48–2.92 (m, 14H, CH_2CH_2Cl, CH_2CH_2CO, CH_2CH_3), 3.67 (s, 6H, OMe), 5.86, 5.92, 6.71 (each s, 1H, –CH=), 8.20 (br s, 3H, NH). λ_{max} 370 nm (ϵ 42,500), 636 (14,500).

2,17 - Bis - (2 - chloroethyl) - 21,24 - dihydro - 8, 12 - bis-(2 - methoxycarbonylethyl) - 3,7,13,18 - tetramethylbilin-1,19 - dione (**14**)

This bilindione was similarly prepared from the b - bilene hydrobromide (**4**) (114 mg). The product (40 mg; 42%) was

crystallized from dichloromethane/hexane and had m.p. 234–235°. Found: C, 60.79; H, 5.89; N, 7.86. $C_{35}H_{40}Cl_2N_4O_6 \cdot \frac{1}{2}H_2O$ requires: C, 60.69; H, 5.92; N, 8.09%. δ, ppm, 1.83, 2.06, 2.07, 2.12 (each s, 3H, Me), 2.50–3.65 (m, 16H, CH_2CH_2Cl, CH_2CH_2CO), 3.67 (s, 6H, OMe), 5.85, 5.89, 6.73 (each s, 1H, –CH=), 8.25, (br s, 3H, NH). λ_{max} 372 nm (ϵ 42,000), 654 (11,600).

2 - *Ethyl* - 21,24 - *dihydro* - 8,12 - *bis* - (2 - *methoxycarbonylethyl*) - 3,7,13,18 - *tetramethyl* - 17 - *vinylmethylbilin* - 1,19 - *dione* (**2**)

The chloroethylbiliverdin (**19**) (35 mg) in degassed pyridine (30 ml) was heated under reflux and under nitrogen for 15 min before 3% aqueous sodium hydroxide (degassed) (3 ml) was added. The mixture was refluxed for 2.5 h and then, after cooling, was treated with 20% acetic acid (5 ml). The mixture was evaporated to dryness (using a toluene chaser) and water (50 ml) was added; the blue precipitate was filtered off, dried under vacuum, and then set aside for 10 h in 5% sulfuric acid/methanol (20 ml). The mixture was poured into aqueous sodium acetate (50 ml), extracted with chloroform (3 × 20 ml), washed with water, aqueous sodium chloride, and water again, and then dried (Na_2SO_4). The solvent was evaporated and the product was purified using preparative thick layer plates of silica gel (elution with 5% methanol in dichloromethane). After removal from the silica gel, the product was crystallized from dichloromethane/hexane to give 25 mg (76%), m.p. 212–215°. Found: C, 68.37; H, 6.84; N, 8.99. $C_{35}H_{40}N_4O_6$ requires: C, 68.61; H, 6.58; N, 9.14%. δ, ppm, 1.08 (t, 3H, CH_2CH_3), 1.85, 2.06, 2.08, 2.12 (each s, 3H, Me), 2.30 (q, 2H, CH_2CH_3), 2.56, 2.85 (each t, 4H, CH_2CH_2CO), 3.67 (s, 6H, OMe), 5.85, 6.05, 6.75 (each s, 1H, –CH=), 5.58–5.68 (q, 2H, vinyl $=CH_2$), 6.52–6.72 (q, 1H, vinyl –CH=), 8.25 (br s, 3H, NH). λ_{max} 370 nm (ϵ 41,000), 650 (13,200).

17 - *Ethyl* - 21,24 - *dihydro* - 8,12 - *bis* - (2 - *methoxycarbonylethyl*) - 3,7,13,18 - *tetramethyl* - 2 - *vinylbilin*-1,19 - *dione* (**3**)

This bilindione was likewise prepared from the 2 - chloroethylbilindione (**24**) (35 mg scale) and gave 23 mg (69%) of the product, m.p. 210–213°. Found: C, 68.32; H, 6.94; N, 8.94. $C_{35}H_{40}N_4O_6$ requires: C, 68.61; H, 6.58; N, 9.14%. δ, ppm, 0.90 (t, 3H, CH_2CH_3), 1.78, 2.11, 2.20, (each s, 3H, 6H, 3H, Me), 2.50–2.95 (m, 10H, CH_2CH_3, CH_2CH_2CO), 3.67 (s, 6H, OMe), 5.99, 6.05, 6.32 (each s, 1H, –CH=), 5.42–5.46, 6.12–6.18 (each d, 1H, vinyl $=CH_2$), 6.48–6.58 (q, 1H, vinyl –CH=), 8.10 (br s, 3H, NH). λ_{max} 373 nm (ϵ 41,800), 654 (13,400).

21,24 - *Dihydro* - 8,12 - *bis* - (2 - *methoxycarbonylethyl*)-3,7,13,8 - *tetramethyl* - 2,17 - *divinylbilin* - 1,19 - *dione* (**1**) ("*biliverdin - IXα dimethyl ester*")

This biliverdin was likewise prepared from the bis - chloroethylbilindione (**14**) (15 mg) and was obtained in 54% yield, m.p. 204–206° (lit[13] m.p. 208–209°). δ, ppm, 1.84, 2.05, 2.12, 2.20 (each s, 3H, Me), 2.5–2.9 (m, 8H, CH_2CH_2CO), 3.67 (s, 6H, OMe), 5.94, 6.02, 6.72 (each s, 1H, –CH=), 5.40–5.62, 5.96–6.08, (m, 4H, vinyl $=CH_2$), 6.48–6.60 (m, 2H, vinyl–CH=), 8.25 (br s, 3H, NH). λ_{max} 381 nm (ϵ 44,000), 642 (14,500).

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REFERENCES

[1]Part VI: K. M. Smith and D. Kishore, *Tetrahedron* **39**, 1841 (1983).
[2]K. M. Smith and D. Kishore, *J. Chem. Soc., Chem., Commun.* 888 (1982).
[3]A. H. Jackson and K. M. Smith, In *Total Syntheses of Natural Products*, (Edited by J. W. ApSimon), Vol. 1, p. 143, Wiley, New York (1973).
[4]S. B. Brown, A. J. Holroyd, R. F. Troxler and G. D. Offner, *Biochem. J.* **194**, 137 (1981).
[5]H. - P. Kost, E. Benedikt, *Z. Naturforsch.* **37C**, 1057 (1982).
[6]J. A. S. Cavaleiro, A. M. d'A. Rocha Gonsalves, G. W. Kenner, and K. M. Smith, *J. Chem. Soc.*, Perkin Trans. I 2471 (1973).
[7]A. H. Jackson, H. A. Sancovich, and A. M. Ferramola de Sancovich, *Bio-org. Chem.* **9**, 71 (1980).
[8]A. W. Johnson, I. T. Kay, E. Markham, R. Price and K. B. Shaw, *J. Chem. Soc.* 3416 (1959).
[9]G. Buldain, J. Hurst, R. B. Frydman and B. Frydman, *J. Org. Chem.* **42**, 2953 (1977).
[10]K. M. Smith, G. W. Craig, F. Eivazi and Z. Martynenko, *Synthesis* 493 (1980).
[11]K. M. Smith and R. K. Pandey, manuscript in preparation.
[12]T. T. Howarth, A. H. Jackson and G. W. Kenner, *J. Chem. Soc.*, Perkin Trans. I 502 (1974).
[13]R. Bonnett and A. F. McDonagh, *J. Chem. Soc.*, Chem. Commun. 238 (1970).