Pergamon

Tetrahedron Vol. 51, No. 8, pp. 2255-2266, 1995

0040-4020/95 $9.50+0.00

0040-4020(94)01103-6

Synthesis of Biliverdins with Stable Extended Conformations. Part II.

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Abstract: The synthesis of two hexacyclic and one heptacyclic biliverdin with extended conformations was achieved using base catalyzed intramolecular substitution reactions of 2-chloroethyl biliverdins. The 2-chloroethyl residues were located at selected β-pyrrole positions as to enable them to react with proximal basic nitrogens at the adjacent pyrrole rings. Seven membered rings were thus formed which distorted either two or the three exocyclic double bonds at the biliverdin meso-bridges away from their usual Z-*syn* configuration. The hexacyclic biliverdin **9** is isomorphous with the chromophores of C-phycocyanin, biliverdin **19** is an isomer of isophorcabilin, and the heptacyclic biliverdin **34** has the fullest extended conformation that the biliverdin backbone can achieve.

INTRODUCTION

In the preceding paper[1] we have shown that on treatment of 2-chloroethyl biliverdins with base intramolecular substitution reactions take place, provided the 2-chloroethyl residues are not located at the pyrrolinone (lactam) rings. Biliverdins with stable extended conformations were then obtained, where ethyl linkages bridge the span between a β-pyrrole carbon and the nitrogen atom of the neighbouring pyrrole ring. These linkages force the biliverdins away from their preferred helical conformations[2,3] which in free biliverdins result from the all-Z-all-*syn* configurations at their exocyclic double bonds. The regioselective synthesis of biliverdins with stable extended conformations at any of their three meso bridges opened the possibility of probing into their biological properties.

In the wing membranes of Lepidoptera biliverdins held in extended conformations by intramolecular bridges (as in phorcabilin and sarpedobilin), could serve as protective pigments[4], or in photoreceptive functions[5]. Biliproteins such as phycobiliproteins keep their tetrapyrrolic chromophores in the Z-*syn* configuration only at the central C(10) meso bridge, while they have extended (Z-*anti*) geometries at the C(5) and the C(15) meso bridges[6]. The latter could be involved in the photodynamic equilibrium of phytochrome[7]. Using partially and fully extended biliverdins we were able to study the regioselectivity of the biliverdin-apomyoglobin recombination at the apomyoglobin crevice[8], as well as the mechanism of the biliverdin reductase[9], the bilirubin forming enzyme. It was also possible to establish that in biliverdins the basicity and the rate of nucleophilic additions at C(10) increase with the stretching of the biliverdin backbone, and that these effects determine the rates of their biological and chemical reductions[10].

We report below the synthetic procedures used to prepare several of the extended biliverdins used in the aforementioned studies. Biliverdin **9** is a partially extended hexacyclic biliverdin isomorphous with the

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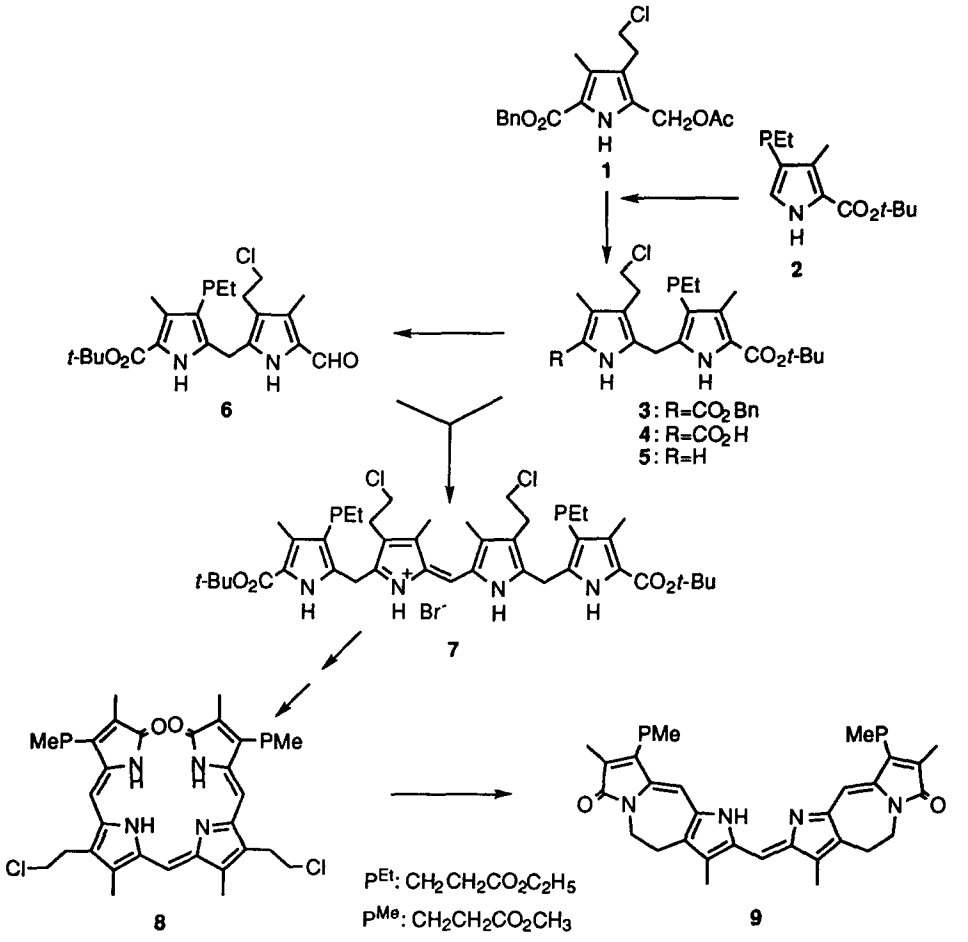
phycocyanobilins of the C-phycocyanins; biliverdin **19** (neobiliverdin IXγ) is an hexacyclic biliverdin isomeric with isophorcabilin, a pigment formed on acid treatment of phorcabilin. Biliverdin **34** is an heptacyclic biliverdin with a completely extended conformation.

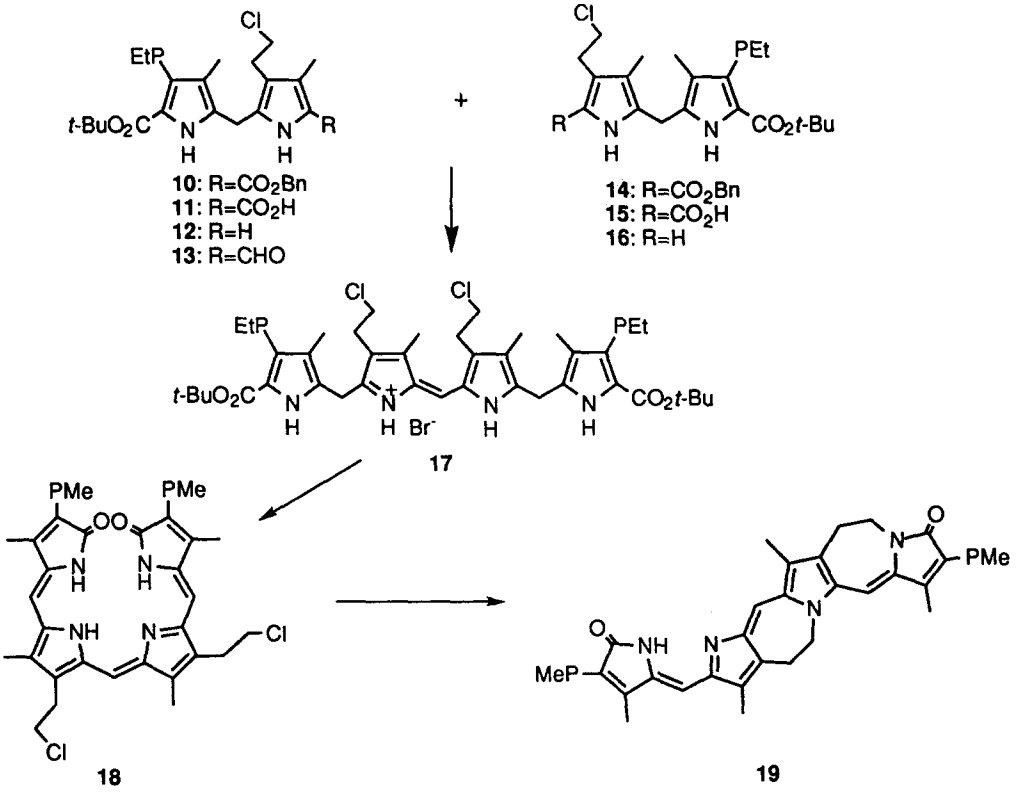
RESULTS AND DISCUSSION

The synthesis of extended biliverdins carrying six rings was achieved following the outline described for the synthesis of pentacyclic biliverdins[1]. A symmetric hexacyclic biliverdin **9** was obtained starting with dipyrrylmethane **3** (Scheme I). The latter was reduced to dipyrrylmethane **4** and decarboxylated to dipyrrylmethane **5**, which was then transformed into its formyl derivative **6** using a Vilsmeier reaction. Condensation of dipyrrylmethanes **5** and **6** afforded the bilene-b hydrobromide **7**, which was oxidized to biliverdin **8** and finally transformed to biliverdin **9**.



Scheme I

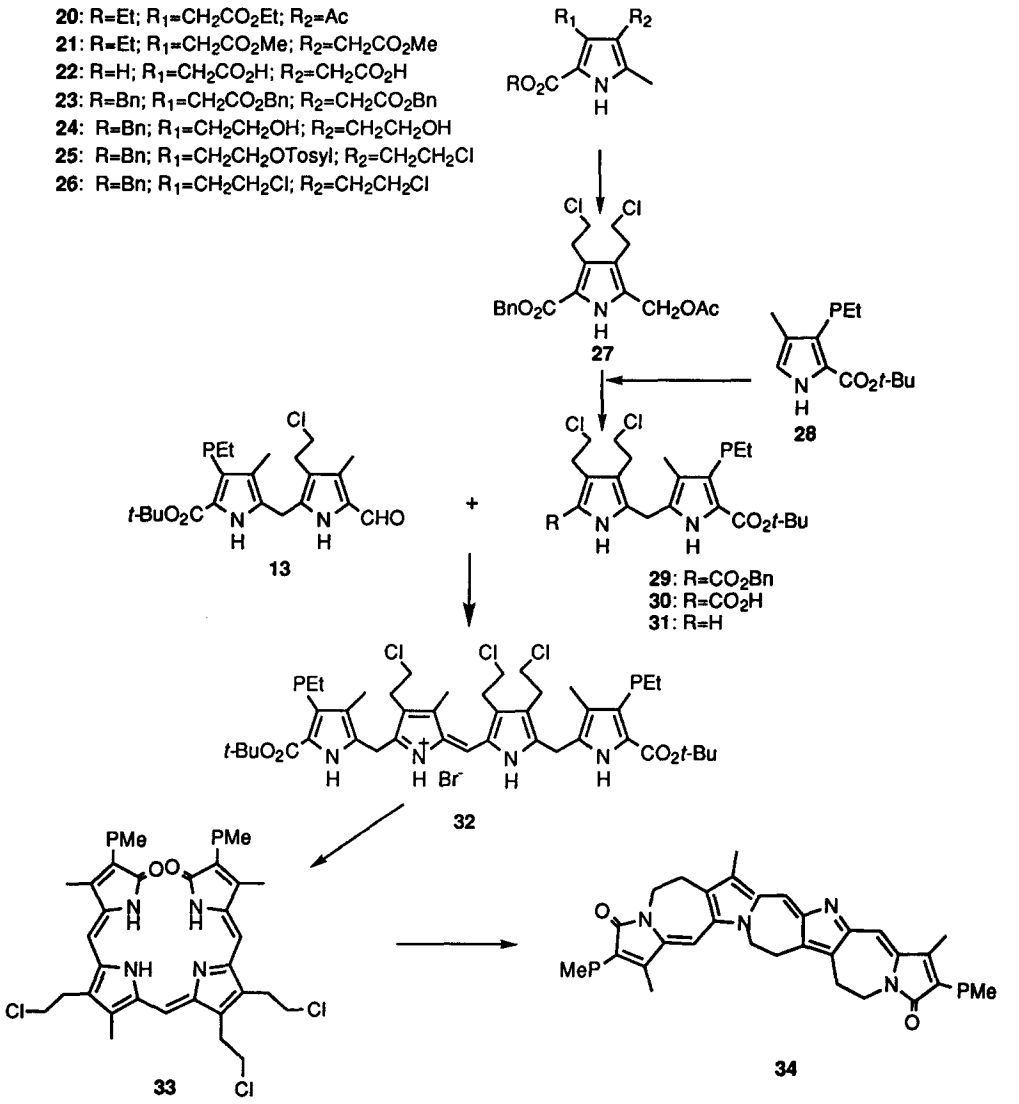
The asymmetric hexacyclic biliverdin **19** (type IXγ) was obtained by condensation of formyl dipyrrylmethane **13** with the α-free dipyrrylmethane **16** (Scheme II). The resulting bilene-b **17** was oxidized to biliverdin **18** and finally converted to biliverdin **19** under basic conditions. The synthesis of the extended biliverdins **9** and **19** lends further support to the reaction pattern outlined in our former report.



Scheme II

Finally, the synthesis of an heptacyclic biliverdin was also achieved (Scheme III). An intermediate bilene-b with three 2-chloroethyl residues was required. It was necessary to place two of them at one pyrrole unit. For this purpose the known pyrrole **20** was oxidized and rearranged with thallium (III) nitrate to give the dimethoxycarbonylmethyl pyrrole **21**. It was then saponified to the triacid **22**, esterified to give the tribenzyl ester **23**, and the latter was reduced with diborane to give the di-(2-hydroxyethyl) pyrrole **24**. The transformation of the pyrrole **24** into the di-(2-chloroethyl) pyrrole **26** could not be achieved in one step; by treatment with tosyl chloride the monochloro-monotosyl derivative **25** was first obtained, the tosylate was then displaced by treatment with lithium chloride. The obtention of the 2-acetoxymethyl pyrrole **27** from **26** was straightforward. It was condensed with pyrrole **28** to give dipyrrylmethane **29** which was then transformed into the α-free dipyrrylmethane **31**. By condensation with **13** it afforded the bilene-b **32**, which was oxidized to the biliverdin **33** and ultimately converted to the fully extended biliverdin **34**. The reaction media for the last step required the

presence of a small quantity of dimethylformamide[11] to enhance the formation of free extended forms; in the absence of DMF partially extended isomers with intact 2-chloroethyl substituents were formed. This biliverdin is almost devoid of UV absorption and absorbs in the visible region, as could be expected from a polyene. It has a deep violet color in the solid state, a very low Rf on silica gel TLC as compared with helical or partially extended biliverdins, and exhibits a strong tendency to add nucleophiles to its C(10) meso bridge[9,10].



Scheme III

EXPERIMENTAL

General : Melting points (m.p.) were determined on a Kofler hot stage apparatus and are uncorrected. UV and visible (UV-Vis) spectra were recorded with a Hitachi U-2000 spectrophotometer; λ_{max} are quoted in nm and band intensities in parentheses, as log ε. ^{1}H-NMR and ^{13}C-NMR spectra were performed in $CDCl_3$ at 80 MHz and 20 MHz respectively with a Varian FT-80A instrument and at 300.13 MHz with a Bruker MSL 300 instrument. Chemical shifts (δ) are given in ppm downfield from tetramethylsilane as internal standard, and observed coupling constants (*J*) in Hertz. Spin multiplicities are indicated by symbols *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet), and *m* (multiplet). ^{1}H and ^{13}C-NMR assignments are based on NOE correlations and Attached Proton Test (APT) and/or chemical shifts. Mass spectra (MS) were obtained in a Shimadzu QP-CG 1000 instrument with EI ionization technique. All chemicals were reagent grade and solvents were distilled prior to use. Thin layer chromatography (TLC) plates (0.25mm thickness, 20x20cm) precoated with silica gel 60 PF_{254} for analytical purposes and silica gel 60 for preparative chromatography were purchased from E. Merck (Darmstadt).

1-Benzyloxycarbonyl-3-(2-chloroethyl)-2,8-dimethyl-7-(2-ethoxycarbonylethyl)-9-tert-butyloxycarbonyl-dipyrrylmethane (**3**). A solution of 699 mg of acetoxymethyl-pyrrole **1**[12] in 50 ml of methylene chloride: methanol 7% was slowly added to a solution of 562 mg of pyrrole **2**[13] and 20 mg of *p*-toluenesulfonic acid in 40 ml of methylene chloride under a stream of nitrogen. The mixture was kept at 40°C during 3 h, after which it was poured over 100 ml of ice-water, the reaction product was extracted with methylene chloride (2x50 ml), the organic layers were pooled, washed with a saturated solution of sodium bicarbonate (2x50 ml), then with water (100 ml) and evaporated to dryness. The residue was dissolved in methylene chloride-hexane 40:60 (v/v) and filtered through a silica gel column (8x4 cm) prepacked, washed and eluted with the same solvent. The eluates containing the dipyrrylmethane **3** were pooled and evaporated to dryness and gave an oily residue; 993 mg (87%) of a light yellow oil were obtained; ^{1}H-NMR, δ: 9.20 and 8.60 (2x*s*, 2H, HN), 7.35 (*s*, 5H, Ph), 5.30 (*s*, 2H, $H_2C(1^3)$), 4.07 (*q*, $^3J_{HH}$=7.2, 2H, $H_2C(7^5)$), 3.95 (*s*, 2H, $H_2C(5)$), 3.55 (*t*, $^3J_{HH}$=6.2, 2H, $H_2C(3^2)$), 3.00-2.45 (*m*, 6H, $H_2C(3^1, 7^1, 7^2)$), 2.34 and 2.28 (2x*s*, 6H, $H_3C(2^1, 8^1)$), 1.57 (*s*, 9H, $H_3C(9^4)$), 1.20 (*t*, $^3J_{HH}$=7.2, 3H, $H_3C(7^6)$); ^{13}C NMR, δ: 173.0 ($C(7^3)$), 161.3 and 161.0 ($C(1^1, 9^1)$), 80.2 ($C(9^3)$), 65.4 ($C(1^3)$), 60.2 ($C(7^5)$), 44.2 ($C(3^2)$), 34.5 ($C(7^2)$), 28.2 ($C(9^4)$), 22.5 ($C(3^1)$), 19.0 ($C(7^1)$), 13.8 ($C(7^6)$), 10.5 and 10.4 ($C(2^1, 8^1)$); MS: 570 (M^+). Anal. calcd. for $C_{31}H_{39}N_2O_6Cl$: C, 65.20; H, 6.88; N, 4.91. Found: C, 65.25; H, 6.83; N, 4.92.

7-(2-Chloroethyl)-2,8-dimethyl-3-(2-ethoxycarbonylethyl)-1-tert-butyloxycarbonyl-dipyrrylmethane (**5**). Dipyrrylmethane **3** (993 mg) was dissolved in 100 ml of tetrahydrofuran containing 0.3 ml of triethylamine and was reduced with hydrogen during 2 h at 50 psi over 150 mg of 10% Pd on charcoal. The catalyst was filtered, the solution was evaporated to dryness in vacuo and the acid **4** thus obtained (769 mg, 92%) was dissolved into 150 ml of dry methylene chloride-methanol (90:10). *p*-Toluenesulfonic acid (770 mg) was added and the mixture

was stirred during 2 h at 20°C. It was then washed with water (100 ml), 5% sodium bicarbonate (100 ml) and again with water (100 ml); the organic layer was evaporated to dryness, the residue was dissolved in a small volume of methylene chloride-hexane 40:60, adsorbed on a silica gel column (4x4 cm), and the dipyrrylmethane **5** was eluted with the same solvent; 636 mg (91%) of a pink oil were obtained; ^{1}H-NMR, δ: 6.40 (broad *s*, 1H, HC(9)), 4.15 (*q*, $^3J_{HH}$=7.2, 2H, $H_2C(3^5)$), 3.90 (*s*, 2H, $H_2C(5)$), 3.60 (*t*, $^3J_{HH}$=6.1, 2H, $H_2C(7^2)$), 3.05-2.35 (*m*, 6H, $H_2C(3^1, 3^2, 7^1)$), 2.28 (*s*, 3H, $H_3C(2^1)$), 2.08 (*d*, $^4J_{HH}$=1.0, 3H, $H_3C(8^1)$), 1.56 (*s*, 9H, $H_3C(1^4)$), 1.27 (*t*, $^3J_{HH}$=7.2, 3H, $H_3C(3^6)$); MS: 436 (M^+). Anal. calcd. for $C_{23}H_{33}N_2O_4Cl$: C, 63.22; H, 7.61; N, 6.41. Found: C, 63.25; H, 7.14; N, 6.45.

7-(2-Chloroethyl)-2,8-dimethyl-3-(2-ethoxycarbonylethyl)-9-formyl-1-tert-butyloxycarbonyl-dipyrrylmethane (**6**). A solution of 400 mg of dipyrrylmethane **5** in 14 ml of dimethylformamide was kept at 5°C and 129 mg of benzoyl chloride were added in one portion. The mixture was kept at 20°C during 1 h, then diluted with ethyl ether (20 ml), and the mixture was extracted with water (3x5 ml). The water extracts were washed once with ethyl ether (10 ml), and the aqueous solution was adjusted to pH 8 with a 10% sodium bicarbonate solution and left during 20 h at 20°C. The oily precipitate was extracted into chloroform (3x10 ml), the organic extracts were evaporated to dryness, and the residue was crystallized from methanol-water; 358 mg (84%); m.p.: 49-50°C (methanol-water); ^{1}H-NMR, δ: 9.5 (*s*, 1H, $HC(9^1)$), 8.00 (*s*, 2H, HN), 4.17 (*q*, $^3J_{HH}$=7.0, 2H, $H_2C(3^5)$), 4.00 (*s*, 2H, $H_2C(5)$), 3.55 (*t*, $^3J_{HH}$=5.8, 2H, $H_2C(7^2)$), 3.10-2.40 (*m*, 6H, $H_2C(3^1, 3^2, 7^1)$), 2.33 and 2.27 (2x*s*, 6H, $H_3C(2^1, 8^1)$), 1.57 (*s*, 9H, $H_3C(1^4)$), 1.24 (*t*, $^3J_{HH}$=7.0, 3H, $H_3C(3^6)$). Anal. calcd. for $C_{24}H_{33}N_2O_5Cl$: C, 61.99; H, 7.15; N, 6.03. Found: C, 62.09; H, 7.19; N, 6.06.

7,13-Di-(2-chloroethyl)-3,17-di-(2-ethoxycarbonylethyl)-1,19-di-tert-butyloxycarbonyl-2,8,12,18-tetramethyl-bilene-b hydrobromide (**7**). Dipyrrylmethane **5** (200 mg) was dissolved in 4 ml of 50% of dry methanol in dry methylene chloride and 213 mg of the formyl dipyrrylmethane **6** were added followed by 0.3 ml of 33% hydrobromic acid in glacial acetic acid. The mixture was kept in the dark at 20°C during 15 min with occasional stirring. It was then adsorbed on a column (1.5x20 cm) of neutral alumina (grade III) prewashed with methylene chloride, the orange main band was eluted with the same solvent and the eluates were collected over 25 ml of dry methanol containing 0.25 ml of 33% hydrobromic acid in glacial acetic acid. The solution was evaporated to dryness in vacuo, redissolved in 50 ml of dry benzene and evaporated to dryness again. The operation was repeated three times when brilliant red crystals were obtained; 410 mg (54%); m.p.: above 300°C ; ^{1}H-NMR, δ: 13.45, 9.89, 8.79 (3x*s*, 4H, HN); 7.18 (*s*, 1H, HC(10)), 4.56-2.45 (*m*, 20 H, $H_2C(3^1, 3^2, 3^5, 7^1, 7^2, 13^1, 13^2, 17^1, 17^2, 17^5)$), 2.24 and 2.01 (2x*s*, 12H, $H_3C(2^1, 8^1, 12^1, 18^1)$), 1.58 (*s*, 18H, $H_3C(1^4, 19^4)$), 1.25 (*t*, $^3J_{HH}$=7.0, 6H, $H_3C(3^6, 17^6)$). Anal. calcd. for $C_{47}H_{64}Cl_2N_4O_8$.HBr: C, 58.51; H, 6.79; N, 5.81. Found: C, 58.59; H, 6.70; N, 5.90.

7,13-Di-(2-chloroethyl)-3,17-di-(2-methoxycarbonylethyl)-2,8,12,18-tetramethyl-bilin-1,19-dione (**8**). The bilene-b hydrobromide **7** (410 mg) was dissolved in 50 ml of trifluoracetic acid under a stream of nitrogen and was kept at 20°C during 10 min, it was then cooled to 5°C and 1.2 ml of bromine were added in six portions

during 1 h. After additional 1.5 h at 5°C the solution was poured over 100 g of sodium bicarbonate while stirring with nitrogen. The mixture was then partitioned between water (200 ml) and chloroform (200 ml), the organic layer was separated, washed with water (2x200 ml), the green solution was dried (Na_2SO_4), filtered and evaporated to dryness. The residue was dissolved in 5% sulfuric acid in dry methanol, the solution was kept in the dark for 18 h, after which it was partitioned between 100 ml of chloroform and 100 ml of water, the organic layer was separated, washed with water (100 ml), then with saturated sodium bicarbonate (100 ml) and finally again with water (100 ml). It was then dried (Na_2SO_4), filtered, evaporated to dryness, the residue was dissolved in a small volume of 5% acetone in chloroform and the solution was filtered through a silica gel column packed and eluted with the aforementioned solvent. The main blue-greenish band was eluted, evaporated to dryness, and the residue crystallized from benzene-hexane into turquoise-blue crystals; 64 mg (21%) were obtained from benzene-hexane; m.p.: 164-166°C; UV-Vis: 634 (4.20), 370 (4.74); ^{1}H-NMR, δ: 6.76 (*s*, 1H, HC(10)), 5.98 (*s*, 2H, HC(5, 15)), 3.69 (*s* , 6H, $H_3C(3^5, 17^5)$), 3.60 (*t*, ^3J=7.3, 4H, $H_2C(7^2, 13^2)$), 2.97 (*t*, ^3J=7.3, 4H, $H_2C(7^1, 13^1)$), 2.83 (*t*, ^3J=7.7, 4H, $H_2C(3^2, 17^2)$), 2.61 (*t*, ^3J=7.7, 4H, $H_2C(3^1, 17^1)$), 2.22 (*s*, 6H, $H_3C(8^1, 12^1)$), 1.83 (*s*, 6H, $H_3C(2^1,18^1)$). Anal. calcd. for $C_{35}H_{40}Cl_2N_4O_6$: C, 61.49; H, 5.90; N, 8.20. Found: C, 61.45; H, 5.80; N, 8.30.

Hexacyclic biliverdin dimethyl ester (**9**). Biliverdin **8** (40 mg) was dissolved in 6 ml of dimethylformamide under a constant stream of nitrogen, and 0.4 ml of 1,8-diazabicyclo [5.4.0] undec-7-ene (DBU) were added. After 2 h at 20°C, methylene chloride (200 ml) was added and the solution was washed with water (3x50 ml). The organic layer was separated, dried (Na_2SO_4), filtered, evaporated to dryness in vacuo, and the residue crystallized from methylene chloride-hexane. Blue prisms were obtained; 12.5 mg (35%); m.p.: 110°C (benzene-heptane); UV-Vis (methanol, pH=7.6); 672 (4.57), 618 (4.48), 375 (4.05); ^{1}H-NMR, δ: 6.75 (*s*, 1H, HC(10)), 6.35 (*s*, 2H, HC(5, 15)), 3.94 (*m*, 4H, $H_2C(7^2,13^2)$), 3.71 (*s*, 6H, $H_3C(3^5,17^5)$), 3.00-2.50 (*m*, 12H, $H_2C(3^1, 3^2, 7^1, 13^1, 17^1, 17^2)$), 2.18 (*s*, 6H, $H_3C(8^1, 12^1)$), 1.99 (*s*, 6H, $H_3C(2^1,18^1)$). Anal. calcd. for $C_{35}H_{38}N_4O_6$: C, 68.84; H, 6.27; N, 9.17. Found: C, 69.01; H, 6.32; N, 9.21.

1-tert-Butyloxycarbonyl-7-(2-chloroethyl)-3,8-dimethyl-2-(2-ethoxycarbonylethyl)-dipyrrylmethane (**12**) was obtained from dipyrrylmethane **10**[14] (650 mg) following the procedure of hydrogenolysis to the acid **11** (510 mg, 93%) and decarboxylation to **12** described for the synthesis of **5**; 449 mg (97%) of a yellow oil were obtained; ^{1}H-NMR, δ: 6.50 (*m*, 1H, HC(9)), 4.20 (*q*, $^3J_{HH}$=7.3, 2H, $H_2C(2^5)$), 3.95 (*s*, 2H, $H_2C(5)$), 3.70 (*t*, $^3J_{HH}$=6.3, 2H, $H_2C(7^2)$), 3.00-2.30 (*m*, 6H, $H_2C(2^1, 2^2, 7^1)$), 2.30 and 2.10 (2x*s*, 6H, $H_3C(3^1,8^1)$), 1.60 (*s*, 9H, $H_3C(1^4)$), 1.20 (*t*, $^3J_{HH}$=7.3, 3H, $H_3C(2^6)$); MS: 436 (M^+). Anal. calcd. for $C_{23}H_{32}N_2O_4Cl$: C, 63.37; H, 7.35; N, 6.43. Found: C, 63.40; H, 7.40; N, 6.51.

1-tert-Butyloxycarbonyl-7-(2-chloroethyl)-3,8-dimethyl-2-(2-ethoxycarbonylethyl)-9-formyl-dipyrrylmethane (**13**) was obtained from **12** (449 mg) by reaction with benzoyl chloride and dimethylformamide following the procedure described for the synthesis of **6**; 340 mg (71%) of a yellow solid were obtained; ^{1}H-NMR, δ: 9.50 (*s*, 1H, $HC(9^1)$), 4.19 (*q*, $^3J_{HH}$=7.0, 2H, $H_2C(2^5)$), 4.00 (*s*, 2H, $H_2C(5)$), 3.55 (*t*, $^3J_{HH}$=6.0,

2H, $H_2C(7^2)$), 3.05-2.45 (*m*, 6H, $H_2C(2^1, 2^2, 7^1)$), 2.33 and 2.26 (2x*s*, 6H, $H_3C(3^1, 8^1)$), 1.56 (*s*, 9H, $H_3C(1^4)$), 1.27 (*t*, $^3J_{HH}$=7.0, 3H, $H_3C(2^6)$). Anal. calcd. for $C_{24}H_{33}N_2O_5Cl$: C, 61.99; H, 7.15; N, 6.03. Found: C, 62.10; H, 7.15; N, 6.09.

1,19-Di-tert-butyloxycarbonyl-7,12-di-(2-chloroethyl)-2,18-di-(2-ethoxycarbonylethyl)-3,8,13,17-tetramethyl-bilene-b hydrobromide (**17**) was obtained following the procedure described for the synthesis of **7**. Dipyrrylmethane **14**[15] (460 mg) was reduced with hydrogen to give the acid **15** (360 mg, 93%), which was decarboxylated to **16** (278 mg, 85%) using *p*-toluenesulfonic acid. The latter was then condensed with 296 mg of formyl-dipyrrylmethane **13**; 497 mg (81%) of the bilene-b hydrobromide were obtained; m.p.: above 300°C. Anal. calcd. for $C_{47}H_{64}Cl_2N_4O_8$.HBr: C, 58.51; H, 6.79; N, 5.81. Found: C, 58.60; H, 6.51; N, 5.76.

7,12-Di-(2-chloroethyl)-2,18-di-(2-methoxycarbonylethyl)-3,8,13,17-tetramethyl-bilin-1,19-dione (**18**) was obtained from 497 mg of bilene-b hydrobromide **17** following the procedure described for **8**; 62 mg (17%) of blue prisms of **18** were obtained; m.p.: 192-194°C (from methylene chloride-heptane); UV-Vis: 662 (4.20), 374 (4.71); ^{1}H-NMR, δ: 6.54 (*s*, 1H, HC(10)), 5.77 and 5.75 (2x*s*, 2H, HC(5, 15)), 3.65 (*s*, 6H, $H_3C(2^5, 18^5)$), 3.58 and 3.54 (2x*t*, ^3J=7.6, 4H, $H_2C(7^2, 12^2)$), 3.00 and 2.91 (2x*t*, ^3J=7.6, 4H, $H_2C(7^1, 12^1)$), 2.51 and 2.50 (2x*s*, 8H, $H_2C(2^1, 2^2$ and $18^1, 18^2)$), 2.17, 2.09, 2.08 and 2.05 (4x*s*, 12H, $H_3C(3^1, 8^1, 13^1, 17^1)$). Anal. calcd. for $C_{35}H_{40}Cl_2N_4O_6$: C, 61.49; H, 5.90; N, 8.20. Found: C, 61.60; H, 5.92; N, 8.10.

Hexacyclic biliverdin dimethyl ester (**19**) (*Neobiliverdin IXγ*) was obtained from 30 mg of biliverdin **18** following the procedure described for the obtention of symmetric hexacyclic biliverdin **9**; 16 mg (62 %) of violet-blue prisms were obtained; m.p.: 201-203°C (benzene-heptane); UV-Vis (methanol, pH=7.6)[10]: 634 (4.30), 379 (3.34); ^{1}H-NMR, δ: 7.35 (*s*, 1H, HC(10)), 6.05 (*s*, 1H, HC(5)), 5.87 (*s*, 1H, HC(15)), 4.20 (*m*, 2H, $H_2C(12^2)$), 3.92 (*m*, 2H, $H_2C(7^2)$), 3.67 and 3.66 (2x*s*, 6H, $H_3C(2^5, 18^5)$), 3.00 (*m*, 2H, $H_2C(12^1)$), 2.84 (*m*, 2H, $H_2C(7^1)$), 2.67 (*m*, 8H, $H_2C(2^1, 2^2, 18^1, 18^2)$), 2.23 (*s*, 3H, $H_3C(8^1)$), 2.19 (*s*, 3H, $H_3C(3^1)$), 2.14 (*s*, 3H, $H_3C(17^1)$), 2.08 (*s*, 3H, $H_3C(13^1)$). Anal. calcd. for $C_{35}H_{38}N_4O_6$: C, 68.84; H, 6.27; N, 9.17. Found: C, 69.00; H, 6.26; N, 9.21.

Ethyl-3,4-di-(2-methoxycarbonylmethyl)-5-methyl-2-pyrrole carboxylate (**21**). Perchloric acid (70%, 28 ml) was slowly added to a solution of 14 g of thallium (III) nitrate in 140 ml of anhydrous methanol kept at 0°C. The mixture was stirred while 10 g of the 4-acetylpyrrole **20**[16] were added and the mixture was kept at 20°C during 5 h. It was then filtered, the filtrate was diluted with water (200 ml), extracted with chloroform (200 ml), the organic layer was then washed with water (150 ml), followed by a 8% sodium bicarbonate solution (150 ml), water again (150 ml), then dried (Na_2SO_4), and finally evaporated to dryness. The oily residue was dissolved in a small volume of chloroform, adsorbed on a silica gel column (6x22 cm), and the pyrrole **21** was eluted with the same solvent; 7g (65%) were obtained; m.p.: 70-72°C (methanol-water).^{1}H NMR: 9.00 (*s*, 1H, HN)), 4.28 (*q*, $^3J_{HH}$=7.4, 2H, $H_2C(2^3)$), 3.87 (*s*, 2H, $H_2C(3^1)$), 3.72 and 3.70 (2x*s*, 6H, $H_3C(3^4, 4^4)$), 3.43 (*s*, 2H, $H_2C(4^1)$), 2.28 (*s*, 3H, $H_3C(5^1)$), 1.38 (*t*, $^3J_{HH}$=7.4, 3H, $H_3C(2^4)$); ^{13}C NMR: 171.4 ($C(3^2, 4^2)$),

160.8(C(2^1)), 59.4 (C(2^3)), 51.1 (C(3^4, 4^4)), 30.3 and 29.5 (C(3^1, 4^1)), 13.8 (C(2^4)), 10.6 (C(5^1)). Anal. calcd. for $C_{14}H_{19}NO_6$: C, 56.56; H, 6.44; N, 4.71. Found: C, 56.60; H, 6.49; N, 4.80.

Benzyl-3,4-di-(2-benzyloxycarbonylmethyl)-5-methyl-2-pyrrole carboxylate (**23**). Pyrrole **21** (7 g) was added to 40 ml of 1N sodium hydroxide and 20 ml of ethanol, and the mixture was stirred in an open flask at 100°C until evaporated to dryness. The residue was dissolved in 30 ml of water, the solution was adjusted to pH 3 with hydrochloric acid, the precipitated acid **22** (3.9 g, 68%) was filtered, dried, dissolved in a mixture of dry dimethylformamide and 50 ml of dry triethylamine, 50 ml of benzyl chloride were slowly added, and the solution was kept at 20°C during 72 h. It was then evaporated to dryness in vacuo, the residue was dissolved in a small volume of chloroform, adsorbed on a silica gel column, and the pyrrole **23** was eluted with the same solvent; 5.5g (66%) of white crystals were obtained; m.p.: 82-84°C (methanol-water); ^{1}H-NMR, δ: 8.75 (*s*, 1H, HN), 7.25 (*s*, 15H, Ph), 5.18 (*s*, 2H, $H_2C(2^3)$), 5.02 and 4.98 (2x*s*, 4H, $H_2C(3^4, 4^4)$), 3.87 (*s*, 2H, $H_2C(3^1)$), 3.40 (*s*, 2H, $H_2C(4^1)$), 2.30 (*s*, 3H, $H_3C(5^1)$). Anal. calcd. for $C_{31}H_{29}NO_6$: C, 77.09; H, 4.81; N, 2.31. Found: C, 76.95; H, 4.77; N, 2.12.

Benzyl-3,4-di-(2-hydroxyethyl)-5-methyl-2-pyrrole carboxylate (**24**). Dry tetrahydrofuran (100 ml) previously saturated with diborane was slowly added to a stirred solution of 11 g of pyrrole **23** in 100 ml of dry tetrahydrofuran kept at 5°C. The mixture was kept at 20°C during 24 h, and the reaction was completed by heating at 40°C during 1 h. Excess diborane was then destroyed with methanol (30 ml), the mixture was evaporated to dryness in vacuo, the residue was redissolved in a small volume of chloroform, adsorbed on a silica gel column (5x10 cm), the latter was washed with chloroform, and the pyrrole **24** was eluted with 3% methanol in chloroform. The oily residue was redissolved in chloroform and the organic layer was repeatedly washed with water to eliminate contaminating boranes, the organic solvent was evaporated to dryness, and the residue was recrystallized with benzene-cyclohexane; 3.3g (50%); m.p.: 108-109°C; ^{1}H-NMR, δ: 8.90 (*s*, 1H, HN), 7.35 (*s*, 5H, Ph), 5.27 (*s*, 2H, $H_2C(2^3)$), 3.70 and 3.60 (2x*t*, 4H, $H_2C(3^2, 4^2)$), 3.00 (*t*, $^3J_{HH}$=5.9, 2H, $H_2C(3^1)$), 2.68 (*t*, $^3J_{HH}$=5.8, 2H, $H_2C(4^1)$), 2.23 (*s*, 3H, $H_3C(5^1)$); ^{13}C NMR, δ: 160.9 (C(2^1)), 65.5 (C(2^3)), 63.2 and 62.5 (C(3^2, 4^2)), 28.3 (C(3^1)), 27.1 (C(4^1)), 11.2 (C(5^1)). Anal. calcd. $C_{17}H_{21}NO_4$: C, 67.31; H, 6.98; N, 4.62. Found: C, 67.30; H, 6.90; N, 4.58.

Benzyl-3,4-di-(2-chloroethyl)-5-methyl-2-pyrrole carboxylate (**26**). Pyrrole **24** (3 g) was dissolved in 50 ml of dry benzene and 10 ml of anhydrous pyridine, and 3 g of *p*-toluenesulfonyl chloride were added. The mixture was stirred at 60°C during 18 h, it was then diluted with methylene chloride (70 ml), washed with water (3x50 ml), the organic layer was evaporated to dryness, the residue was dissolved in a small volume of chloroform and adsorbed on a silica gel column (2x22 cm) packed and prewashed with the same solvent. The column was eluted with chloroform, the fastest running compound was the di-(2-chloroethyl) derivative **26** (Rf: 0.8 on TLC silica gel), the slowest was the 3-(2-tosylethyl) pyrrole **25**; 1g (21%); m.p.: 98-100°C (methanol-water); ^{1}H-NMR, δ: 8.95 (*s*, 1H, HN), 7.43 (*dd*, 4H, $HC(3^6, 3^{6'}, 3^7, 3^{7'})$), 7.37 (*s*, 5H, Ph), 5.21 (*s*, 2H, $H_2C(2^3)$), 4.14 (*t*, $^3J_{HH}$=5.8, 2H, $H_2C(3^2)$), 3.46 (*t*, $^3J_{HH}$=6.0, 2H, $H_2C(4^2)$), 3.01 (*t*, $^3J_{HH}$=5.8, 2H,

$H_2C(3^1)$), 2.76 (*t*, $^3J_{HH}$=6.0, 2H, $H_2C(4^1)$), 2.41 (*s*, 3H, $H_3C(3^9)$), 2.21 (*s*, 3H, $H_3C(5^1)$). Anal. calcd. $C_{24}H_{26}NO_5S$: C, 60.57; H, 5.47; N, 2.94. Found: C, 60.65; H, 5.51; N, 3.01. Pyrrole **25** (1 g) was dissolved in 50 ml of dry methanol, 3 g of lithium chloride and 1.5 g of ammonium chloride were added and the mixture was stirred for 18 h at 65°C. It was then poured into 200 ml of water, the aqueous solution was extracted with chloroform (2x50 ml), the latter was washed with water, dried (Na_2SO_4) and evaporated to dryness. The residue, pyrrole **26**, was pooled with the product obtained in the former preparation; it was then dissolved in a small volume of chloroform, adsorbed on a silica gel column packed and prewashed with chloroform and eluted with the same solvent; 1.8 g (58%); m.p.: 136-137°C; ^{1}H-NMR, δ: 8.80 (*s*, 1H, HN), 7.32 (*s*, 5H, Ph), 5.34 (*s*, 2H, $H_2C(2^3)$), 3.61 (*m*, 4H, $H_2C(3^2, 4^2)$), 3.17 (*t*, $^3J_{HH}$=6.8, 2H, $H_2C(3^1)$), 2.91 (*t*, $^3J_{HH}$=6.0, 2H, $H_2C(4^1)$), 2.30 (*s*, 3H, $H_3C(5^1)$); ^{13}C NMR, δ: 65.9 ($C(2^3)$), 44.3 ($C(3^2, 4^2)$), 28.8 ($C(3^1)$), 27.6 ($C(4^1)$), 11.5 ($C(5^1)$). Anal. calcd. $C_{17}H_{19}Cl_2NO_2$: C, 59.66; H, 6.18; N, 4.09. Found: C, 59.81; H, 6.20; N, 4.12.

Benzyl-5-acetoxymethyl-3,4-di-(2-chloroethyl)-2-pyrrole carboxylate (**27**). Lead tetraacetate (800 mg) was added to a solution of 600 mg of pyrrole **26** in 60 ml of glacial acetic acid. The mixture was stirred at 20°C during 18 h, it was then poured over an excess of ice-water, filtered, dried and crystallized from methanol; 411 mg (59%); m.p.: 115°C; ^{1}H-NMR, δ: 9.36 (*s*, 1H, HN), 7.37 (*s*, 5H, Ph), 5.31 (*s*, 2H, $H_2C(2^3)$), 5.04 (*s*, 2H, $H_2C(5^1)$), 3.60 (*m*, 4H, $H_2C(3^2, 4^2)$), 3.15 (*t*, $^3J_{HH}$=6.3, 2H, $H_2C(3^1)$), 2.97 (*t*, $^3J_{HH}$=6.3, 2H, $H_2C(4^1)$), 2.1 (*s*, 3H, $H_3C(5^4)$); ^{13}C NMR, δ: 167.45 ($C(5^3)$), 160.1 ($C(2^1)$), 66.1 ($C(2^3)$), 56.7 ($C(5^1)$), 44.1 and 44.5 ($C(3^2, 4^2)$), 28.8 and 28.5 ($C(3^1, 4^1)$), 20.6 ($C(5^4)$). Anal. calcd. $C_{19}H_{21}Cl_2NO_4$: C, 57.30; H, 5.31; N, 3.52. Found: C, 57.41; H, 5.40; N, 3.80.

1-Benzyloxycarbonyl-9-tert-butyloxycarbonyl-2,3-di-(2-chloroethyl)-8-(2-ethoxycarbonyl-ethyl)-7-methyl-dipyrrylmethane (**29**) was obtained by condensation of 440 mg of acetate **27** with pyrrole **28**[17] (350 mg) following the procedure described for **3**. The crude dipyrrylmethane was purified on a silica gel column (2x20 cm) packed and eluted with methylene chloride; 600 mg (77%); m.p.: 150-152°C (methanol-water); ^{1}H-NMR, δ: 9.44 and 9.02 (2x*s*, 2H, HN), 7.32 (*s*, 5H, Ph), 4.11 (*q*, 2H, $H_2C(8^5)$), 3.87 (*s*, 2H, $H_2C(5)$), 3.50 (*m*, 4H, $H_2C(2^2, 3^2)$), 3.15 (*m*, 4H, $H_2C(2^1, 3^2)$), 2.85 (*m*, 2H, $H_2C(8^2)$), 2.50 (*m*, 2H, $H_2C(8^1)$), 1.97 (*s*, 3H, $H_3C(7^1)$), 1.55 (*s*, 9H, $H_3C(9^4)$), 1.26 (*t*, 3H, $H_3C(8^6)$); ^{13}C NMR, δ: 172.9 ($C(8^3)$), 161.0 and 160.7 ($C(1^1, 9^1)$), 80.5 ($C(9^3)$), 65.9 ($C(1^3)$), 59.8 ($C(8^5)$), 43.9 ($C(2^2, 3^2)$), 33.9 ($C(8^2)$), 30.0 ($C(9^4)$), 28.6 ($C(2^1, 3^1)$), 20.8 ($C(8^1)$), 13.9 ($C(8^6)$), 8.3 ($C(7^1)$); MS: 618 (M^+). Anal. calcd. for: $C_{32}H_{40}N_2O_6Cl_2$: C, 62.03; H, 6.46; N, 4.52. Found: C, 62.15; H, 6.50; N, 4.60.

1,19-Di-tert-butyloxycarbonyl-2,18-di-(2-ethoxycarbonylethyl)-7,8,13-tri-(2-chloroethyl)-3,12,17-trimethyl-bilene-b hydrobromide (**32**) was prepared following the obtention described for **7**. Dipyrrylmethane **29** (400 mg) dissolved in methanol (100 ml) was reduced with hydrogen over 10% palladium over charcoal to the acid **30** (328 mg, 96%); the latter was decarboxylated by dissolution in a mixture of 60 ml of methylene chloride and 10 ml of methanol which contained 330 mg of *p*-toluenesulfonic acid, and the a-free

dipyrrylmethane **31** thus obtained (295 mg, 98%) was condensed with 280 mg of formyl dipyrrylmethane **13**; 560 mg (92%) of red crystals of the hydrobromide **32** were thus obtained; m.p.: above 300°C (from benzene-hexane). Anal. calcd. for $C_{48}H_{65}N_4O_8Cl_3.HBr$: C, 56.84; H, 6.65; N, 5.52. Found : C, 56.91; H, 6.60; N, 5.59.

2,18-Di-(2-methoxycarbonylethyl)-7,8,13-tri-(2-chloroethyl)-3,12,17-trimethyl-bilin-1,19-dione (**33**) was obtained from 300 mg of the bilene-b hydrobromide **32** following the procedure described for **8**; 72 mg (32%) of blue prisms were obtained; m.p.: 207-209°C (methylene chloride-heptane); ^{1}H-NMR, δ: 6.59 (*s*, 1H, HC(10)), 5.78 and 5.77 (2x*s*, 2H, HC(5, 15)), 3.66 (*s*, 6H, $H_3C(2^5, 18^5)$), 3.62, 3.58 and 3.55 (3x*t*, $^3J_{HH}$=7.4, 6H, $H_2C(7^2, 8^2, 13^2)$), 3.05, 2.96 and 2.92 (3x*t*, $^3J_{HH}$=7.4, 6H, $H_2C(7^1, 8^1, 13^1)$), 2.55 (*s*, 8H, $H_2C(2^1, 2^2, 18^1, 18^2)$), 2.18 (*s*, 3H, $H_3C(12^1)$, 2.11 (*s*, 6H, $H_3C(3^1, 17^1)$). Anal. calcd. for $C_{36}H_{41}N_4O_6Cl_3$: C, 59.06; H, 5.65; N, 7.65. Found : C, 59.15; H, 5.69; N, 7.71.

Heptacyclic biliverdin dimethyl ester (**34**). Biliverdin **33** (25 mg) was dissolved in 10 ml of dimethylformamide and 2 ml of dimethylsulfoxide and was treated with 10 ml of DBU as described for **9**. The blue solid obtained after evaporation of the solvents was purified using several silica gel TLC plates. The plates were first developed with 8% acetone in chloroform which eluted two fast running bands of partially cyclized biliverdins, followed by 40% acetone in chloroform when the blue band corresponding to **34** reached a Rf ca. 0.5. This band was eluted with 2% dimethylsulfoxide in acetone; after evaporation violet prisms were left behind and were recrystallized from benzene-heptane; 7 mg (32%); UV-Vis (methanol, pH=7.6); 675 (4.40), 377 (3.30); ^{1}H-NMR, δ: 7.50, 6.50 and 6.07 (3x*s*, 3H, HC(5, 10, 15)), 4.50, 4.25, 3.95 (3x*m*, 6H, $H_2C(7^2, 8^2, 13^2)$), 3.71 (*s*, 6H, $H_3C(2^5, 18^5)$), 2.80 (*m*, 6H, $H_2C(7^1, 8^1, 13^1)$), 2.70 (*s*, 8H, $H_2C(2^1, 2^2, 18^1, 18^2)$), 2.29 and 2.21 (2x*s*, 9H, $H_3C(3^1, 12^1, 17^1)$). Anal. calcd. for $C_{36}H_{38}N_4O_6$: C, 69.44; H, 6.15; N, 9.00. Found: C, 69.51; H, 6.16; N, 9.10.

Acknowledgment: Financial support from the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Argentina, is gratefully acknowledged. S. E. B. is grateful to the University of Buenos Aires for a fellowship.

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(*Received in USA* 3 *October* 1994; *accepted* 12 *December* 1994)