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Synthesis of Biliverdins with Stable Extended Conformations. Part I.

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Abstract: Biliverdins with extended conformations stabilized by intramolecular ethyl bridges were obtained by base treatment of helical biliverdins with 2-chloroethyl side chains. Thus, neobiliverdin IXβ (**6**) was obtained by reaction of 13,18-di-(2-chloroethyl)-biliverdin **5** with DBU. During the reaction the 2-chloroethyl-C(13) residue underwent an intramolecular substitution reaction with N-24 while the 2-chloroethyl-C(18) residue underwent an elimination reaction to form a vinyl residue. This reaction scheme was unambiguously demonstrated by performing the synthesis of [^{15}N-24]-dihydro-neobiliverdin IXβ (**19**) and of [^{15}N-23]-dihydrophorcabilin (**31**). The method was then applied to the synthesis of neobiliverdin IXδ, a natural product isolated from the ovaries of the sea snake *Turbo cornutus*. It was concluded that when the 2-chloroethyl side chains are at C(3) (or the equivalent C(17)) and C(2) (or the equivalent C(18)) positions of the biliverdin, elimination reactions lead to vinyl residues in basic media; at any other of the β-pyrrole sites, treatment with base leads to the formation of seven-membered rings by intramolecular substitution reactions.

INTRODUCTION

The dehydrochlorination of 2-chloroethyl residues in the presence of base[1,2] is the method of choice for the obtention of vinyl side chains in the synthesis of biologically relevant porphyrins[3]. The vinyl residues are generated after the tetrapyrrole is assembled. The method was also successfully used during the synthesis of biliverdin IXα[4], but in later work[5] we showed that the dehydrochlorination reaction leads to biliverdins with extended conformations rather than to the expected helical vinyl biliverdins. Further studies were helpful to understand the course of these reactions in the biliverdin series.

Biliverdins are oligopyrroles which usually have the helical all-*syn* conformation (as in **5** (Scheme I)) due to an efficient intrachromophoric hydrogen bond system based on the N(21)-H···N(22)···H-N(23) bonding network. They only adopt stable extended forms in covalently bridged structures[6,7], or when they are part of biliproteins where hydrogen bonds and salt linkages disrupt the central hydrogen bonding system mentioned above[8]. In highly diluted solutions however, short lived partially extended conformers of free biliverdins have been detected[9]. It was therefore conceivable that on treatment with base, two reaction pathways were possible in 2-chloroethyl biliverdins; namely, the dehydrochlorination (elimination) reactions leading to vinyl side chains, or intramolecular substitution reactions between the 2-chloroethyl residue and the basic pyrrolenine or pyrrolinone nitrogens. Given the conformational flexibility of the biliverdin skeleton, the intramolecular substitution reactions should be favoured, except when 2-chloroethyl side chains are located at the pyrrolinone rings and are therefore beyond the reach of the basic nitrogens. As we report below, the substitution reaction pathways are the faster ones and take precedent whenever both reaction centers come into proximity. The

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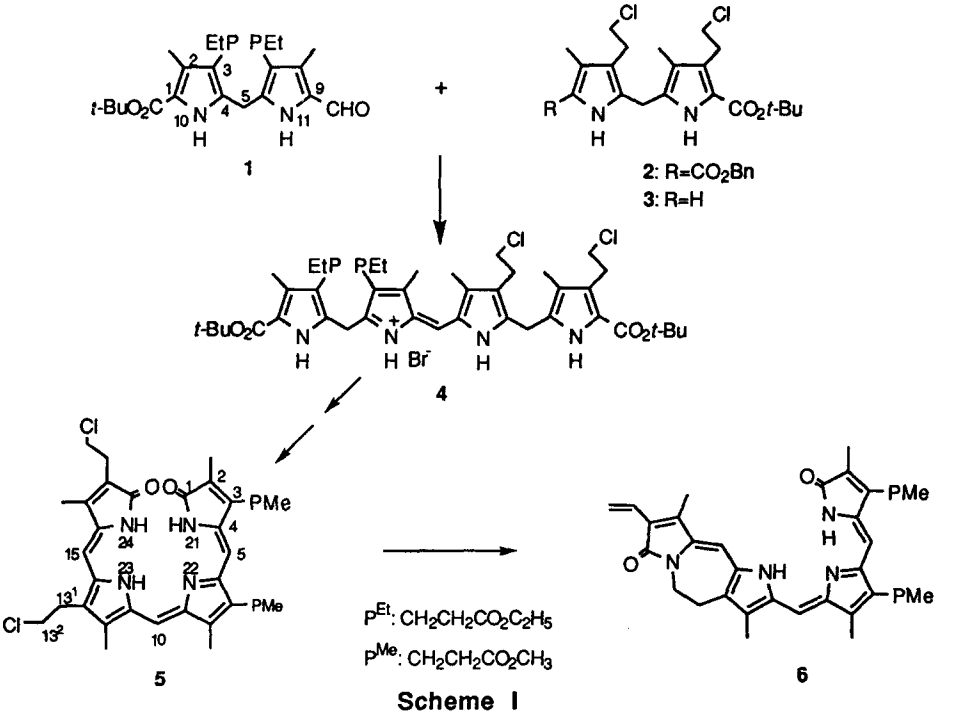
intramolecular reactions between the 2-chloroethyl residues and the basic nitrogens lead to the formation of seven membered rings and force the biliverdin skeleton into an extended conformation. We discuss below the successful use of this approach for the synthesis of several biliverdins with stable extended geometries. By making use of ^{15}N-enriched precursors it was possible to draw unambiguous conclusions as to the reaction course of the intramolecular substitution reactions.

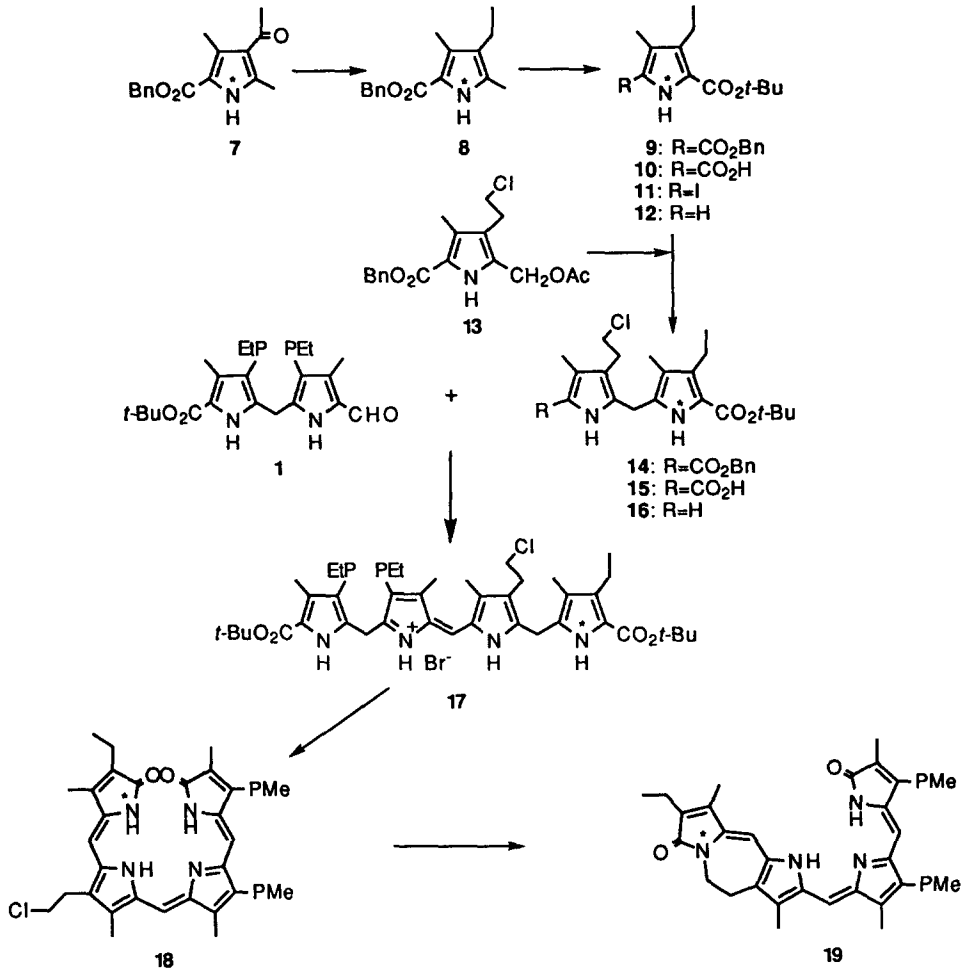
RESULTS AND DISCUSSION

The obtention of a neobiliverdin[10] of type β is outlined in Scheme I. Condensation of the α-free dipyrrylmethane **3** with the formyl dipyrrylmethane **1** gave the di-*tert*-butyloxycarbonyl bilene-b hydrobromide **4**. The latter was hydrolyzed and decarboxylated in trifluoracetic acid and then oxidized with bromine as described elsewhere[11] to afford the helical biliverdin **5**. On treatment with base (DBU, KOH in pyridine or *t*-BuOK in *t*-BuOH) neobiliverdin IXβ (**6**) was obtained. Its UV-vis spectrum ($R^{Vis\ max}/_{UV\ max} = 0.90$) already indicated that it had a partially extended conformation [12].



Scheme I

The NMR spectrum showed the characteristic pattern[13] of an exo vinyl residue formed at expense of the C(18) side chain, but it was not entirely clear how the C(13)-2-chloroethyl residue had reacted. A resonance at 3.93 ppm attributed to one methylene suggested that it was attached to an electronegative atom, and N(24) was obviously the best choice[14]. To put this hypothesis on a firm basis, [^{15}N-24]-dihydroneobiliverdin IXβ (**19**) was prepared (Scheme II).



Scheme II

Starting with [^{15}N]-pyrrole **7** and following classical pyrrole chemistry, [^{15}N]-pyrrole **12** was prepared and brought into reaction with the 2-acetoxymethyl pyrrole **13**[15] to give [^{15}N]-dipyrrylmethane **14**. It was transformed into [^{15}N]-dipyrrylmethane **16** carrying the prospective ring D of biliverdin **19** enriched with ^{15}N. By reaction with formyl dipyrrylmethane **1**, [^{15}N-24]-bilene-b hydrobromide **17** was obtained and was transformed, *via* helical biliverdin **18**, into the expected [^{15}N-24]-biliverdin **19**. The ^{13}C-NMR spectra of **6** and **19** settled the issue of the intramolecular attachment of the C(13) side chain. In biliverdin **6** the H_2C-N resonance at 39.95 ppm was a singlet[5], while in biliverdin **19** the homologous signal at 40.03 ppm was a doublet with a $J_{C\text{-}N}$=9.8 Hz. The 3.93 and 3.95 resonances in the ^{1}H-NMR spectra of biliverdins **6** and **19** were multiplets and corresponded to the $H_2CN(24)$ resonance. The homonuclear COSY and NOESY spectra of neobiliverdin IXβ (**6**) compared with that of biliverdin IXβ (Figure I), confirmed the partially extended geometry of the tetrapyrrole backbone.

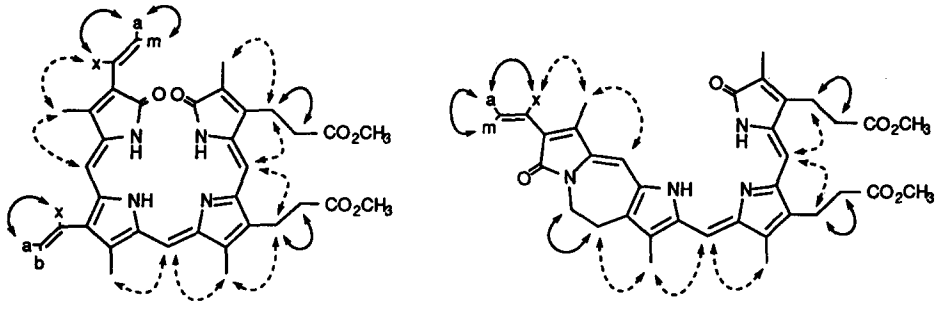
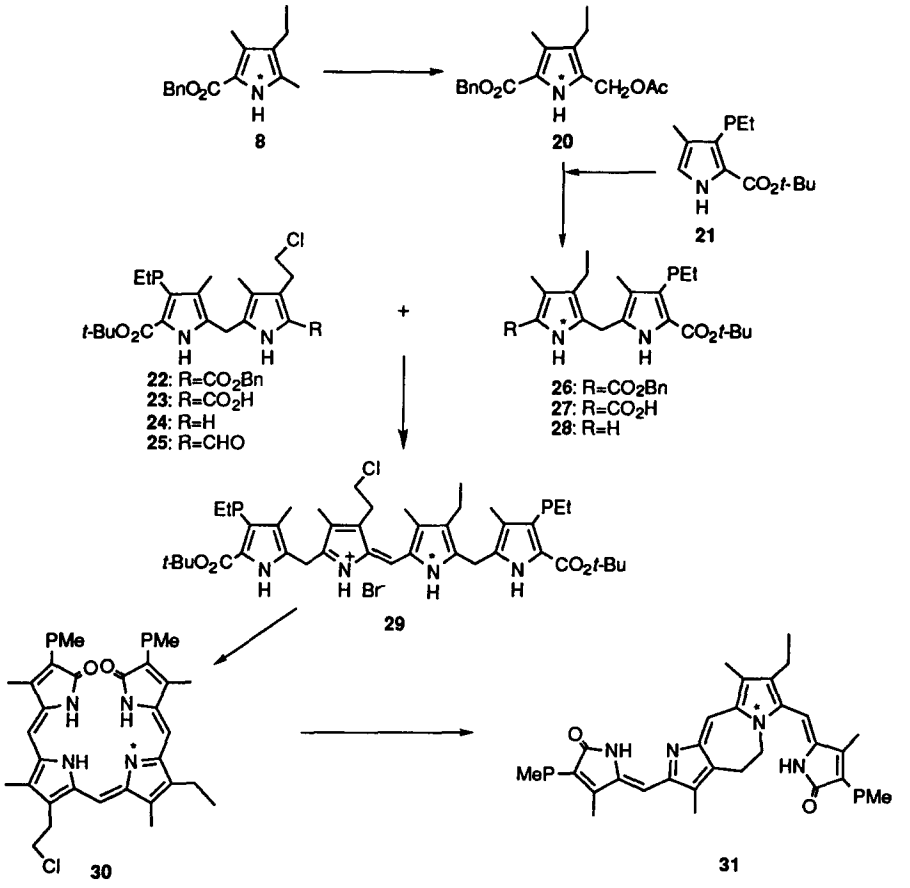


FIGURE I: Homonuclear correlations in COSY (—) and NOESY (---) spectra of biliverdin IX β and neobiliverdin IX β.

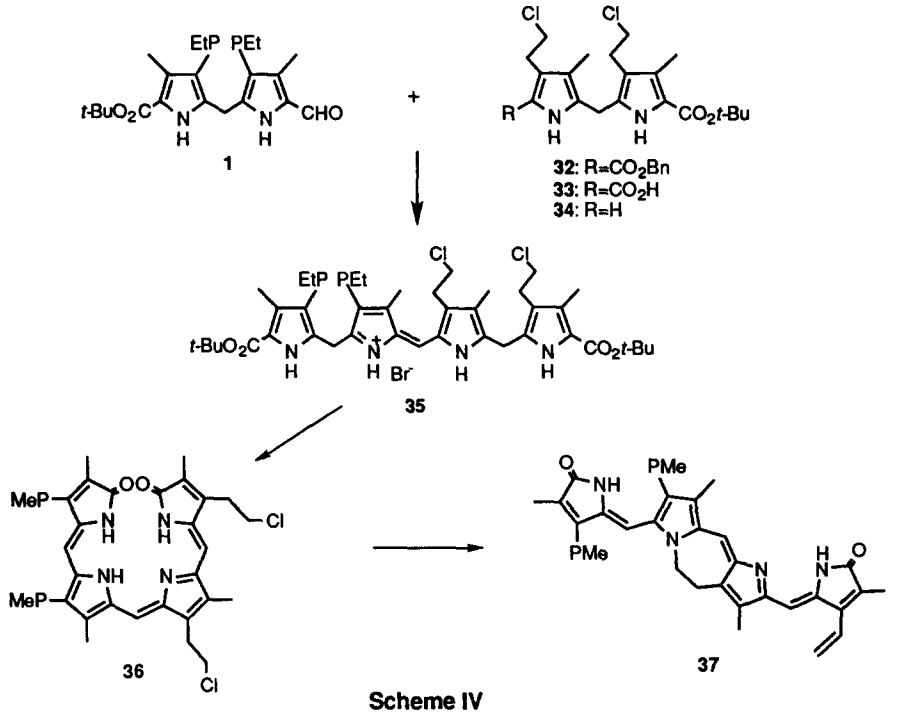
To further test the path of the synthetic approach we decided to prepare [^{15}N-23]-dihydrophorcabilin (**31**). The synthesis of [^{15}N-23]-biliverdin **31** (Scheme III)



Scheme III

was based on the preparation of [^{15}N]-pyrrole **20** which was condensed with pyrrole **21** to first give [^{15}N]-dipyrrylmethane **26**, and then [^{15}N]-dipyrrylmethane **28**. Condensation with dipyrrylmethane **25** gave the bilene-b hydrobromide **29** which was oxidized to biliverdin **30**, and on treatment with base was transformed into [^{15}N-23]-biliverdin **31**. Its ^{1}H-NMR spectrum had a multiplet at 3.90 ppm while the ^{13}C-NMR spectrum showed the coupled 45.55 ppm (J_{CN}=10.2 Hz) resonance.

Neobiliverdin IXδ (**37**) is a natural product isolated from the ovaries of the sea snake *Turbo cornutus*[16]; it can be obtained semisynthetically irradiating biliverdin IXδ in DMSO with visible light[17,18]. The total synthesis of **37** was achieved following the former outlines (Scheme IV). By condensation of dipyrrylmethane **34** with formyl dipyrrylmethane **1**, bilene-b hydrobromide **35** was obtained. The latter was transformed into biliverdin **36**, which on treatment with base gave biliverdin **37**.



Scheme IV

The 2-chloroethyl side chain at C(12) reacted with N(22) only when tautomerism (see Figure II) brought into proximity the reaction centers; the base catalysed migration of double bonds within the pyrrole-pyrrolenine moiety resulted in an overall change in the configuration at the meso C(10) position (Z-*syn* ' E-*anti*) and the desired reaction occurred in high yield. On the other hand, the 2-chloroethyl residue at C(17) underwent elimination to give a vinyl group, as the Z-E change in the configuration required for the intramolecular substitution reaction on N(23) could not take place under the reaction conditions used. The ^{1}H-NMR signal of HC(10) in biliverdin **37** was shifted to lower fields, when compared with HC(10) in biliverdin **36** (from 6.70 to 7.30 ppm). Similar shifts were also observed in photoisomerization products of biliverdin IXα[19].

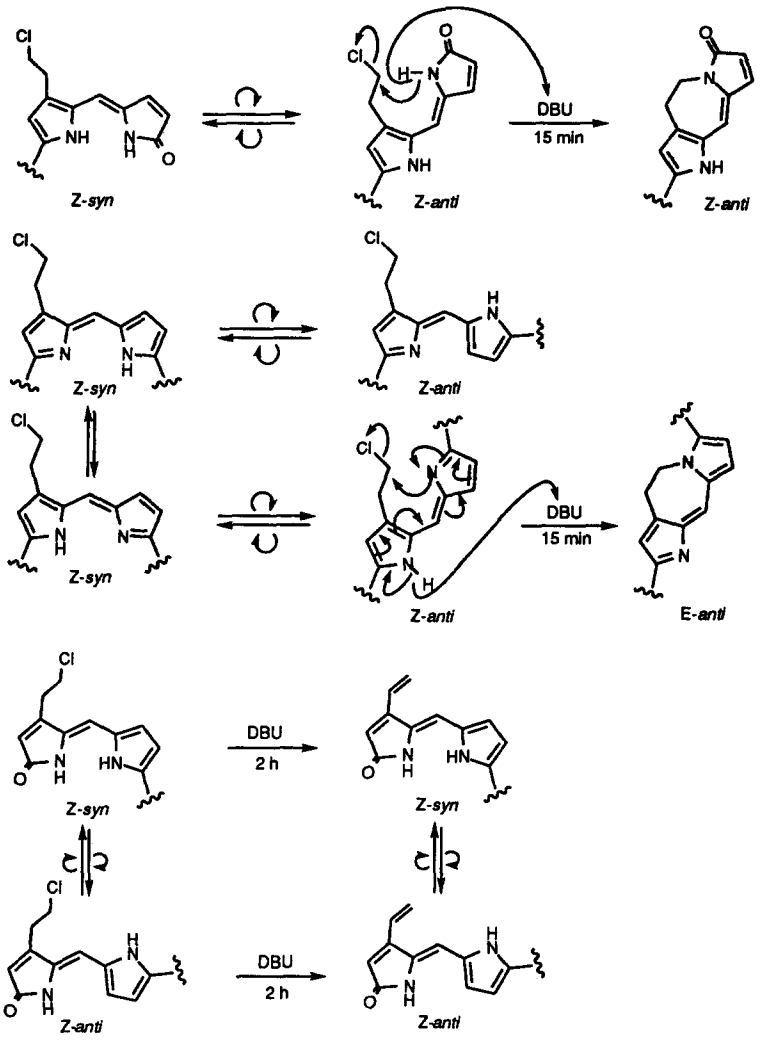


FIGURE II: Conformational flexibility of biliverdins: Z-*syn* dipyrromethenone moieties achieve the desired Z-*anti* conformation by rotation around a single bond, bringing into proximity the reaction centers when the chloroethyl side chains are not placed on a lactam ring. Z-*syn* dipyrrylmethene moieties require both tautomerism at the pyrrole-pyrrolenine rings and rotation around a single bond to place the reaction centers in positions where the intramolecular substitution reactions can take place. An overall configurational change (E-*anti*) then results.

The remarkable high flexibility of the biliverdin skeleton allows concerted intramolecular substitution reactions to take place during the base catalysed dehydrochlorination of 2-chloroethyl biliverdins with formation of seven-membered rings. Since substitution reactions were found to be faster (ca. 15 min) than the elimination reactions (ca. 2 h) seven-membered rings are always formed when the conformation at the meso bridges brings into proximity the reaction centers. Only when the 2-chloroethyl side chains are at the C(3) (or C(17)) and C(2) (or C(18)) positions of the tetrapyrrole backbone, the distances between the 2-chloroethyl residues and the

pyrrolenine nitrogens prevent the substitution reactions; elimination reactions then take place and vinyl residues are formed (Figure II). The reaction outline envisaged in Figure II allowed us to design a synthetic route for other extended biliverdins.

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 using neutral CH_2Cl_2 solutions, unless specified; 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).

1,19-Di-tert-butyloxycarbonyl-3,7-di-(2-ethoxycarbonylethyl)-13,18-di-(2-chloroethyl)-2,8, 12,17-tetramethyl-bilene-b hydrobromide (**4**). Dipyrrylmethane **2** (1.0 g)[15] was dissolved in 150 ml of methanol and was reduced with hydrogen over 0.2 g of 10% palladium on charcoal at 50 psi during 2 h. The catalyst was filtered off, the solution was evaporated to dryness *in vacuo*, the residue was dissolved in 50 ml of methylene chloride, 0.4 g of *p*-toluenesulfonic acid were added and the mixture was kept at 20°C during 2 h. The solution was washed with water (100 ml), then with a 5% sodium bicarbonate solution (100 ml) and finally again with water (100 ml). The organic layer was separated, dried (Na_2SO_4), filtered, and evaporated to dryness *in vacuo*. The residue was dissolved in 2% methanol in benzene and was filtered through a short column packed with silica gel, prewashed and eluted with the same solvent. The eluates containing the dipyrrylmethane **3** were pooled and evaporated to dryness. The residue (510 mg, 68%) was redissolved in 6 ml of 50% of dry methanol in dry methylene chloride and 652 mg of the formyl dipyrrylmethane **1** were added followed by 0.6 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 50 ml of dry methanol containing 0.5 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; 1.1 g (87%); m.p. > 300°C; ^{1}H-NMR, δ: 13.50, 9.90 and 8.85 (3x*s*, 4H, 4xNH), 7.15 (*s*, 1H, HC(10)), 4.5-2.5 (*m*, 24H, $H_2C(3^1, 3^2, 3^5, 5, 7^1, 7^2, 7^5, 13^1, 13^2, 15, 18^1, 18^2)$), 2.30, 2.24, 2.10, 2.00 (4x*s*, 12H, $H_3C(2^1, 8^1, 12^1, 17^1)$), 1.58 (*s*, 18H, $H_3C(1^4, 19^4)$), 1.25 (*t*, 6H, $H_3C(3^6, 7^6)$); MS: 883 $(M+H)^+$. Anal. calcd. for $C_{47}H_{65}N_4O_8Cl_2Br$: C, 58.50; H, 6.74; N, 5.81. Found: C, 58.45; H, 6.61; N, 6.00.

3,7-Di-(2-methoxycarbonylethyl)-13,18-di-(2-chloroethyl)-2,8,12,17-tetramethyl-bilin-1,19-dione (**5**). The bilene-b hydrobromide **4** (440 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 aqueous solution (100 ml) and finally again with water (100 ml). It was then dried (Na_2SO_4), filtered, evaporated to dryness, the residue was redissolved 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 needles; 87 mg (28%); m.p.:197-198°C; UV-Vis: 644 (4.08), 366 (4.62); ^{1}H-NMR, δ: 6.65 (*s*, 1H, HC(10)), 5.94 and 5.84 (2x*s*, 2H, HC(5, 15)), 3.65 (*s*, 6H, $H_3C(3^5, 7^5)$), 3.60 and 3.55 (2x*t*, $^3J_{HH}$=7.3 and 7.4, 4H, $H_2C(13^2, 18^2)$), 2.90 (*t*, $^3J_{HH}$=7.5, 2H, $H_2C(7^1)$), 2.79 (*m*, 4H, $H_2C(3^1, 13^1)$), 2.69 (*t*, $^3J_{HH}$=7.3, 2H, $H_2C(18^1)$), 2.60 (*t*, $^3J_{HH}$=7.5, 2H, $H_2C(3^2)$), 2.47 (*t*, $^3J_{HH}$=7.5, 2H, $H_2C(7^2)$), 2.18, 2.17 and 2.11 (3x*s*, 9H, $H_3C(8^1, 12^1, 17^1)$), 1.80 (*s*, 3H, $H_3C(2^1)$); MS: 682 M^+. Anal. calcd. for $C_{35}H_{40}N_4O_6Cl_2$: C, 61.49; H, 5.90; N, 8.20. Found: C, 61.50; H, 5.60; N, 8.30.

Neobiliverdin IX β (**6**). Biliverdin **5** (87 mg) was dissolved in 12 ml of dimethylformamide under a constant stream of nitrogen, and 0.9 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. Navy blue prisms were obtained; 52 mg (67%); m.p.: 210-212°C; UV-Vis: 662 (4.47), 377 (4.53); ^{1}H-NMR, δ: 6.70 (*s*, 1H, HC(10)), 6.55 (*m*, 1H, $HC(18^1)$), 6.34 (*m*, 1H, $H_mC(18^2)$), 6.33 (*s*, 1H, HC(5)), 5.99 (*s*, 1H, HC(15)), 5.47 (*m*, 1H, $H_aC(18^2)$), 3.93 (*m*, 2H, $H_2C(13^2)$), 3.67 (*s*, 3H, $H_3C(3^5)$), 3.65 (*s*, 3H, $H_3C(7^5)$), 2.91 (*m*, 2H, $H_2C(7^1)$), 2.85 (*m*, 2H, $H_2C(13^1)$), 2.84 (*m*, 2H, $H_2C(3^1)$), 2.62 (*m*, 2H, $H_2C(3^2)$), 2.53 (*m*, 2H, $H_2C(7^2)$), 2.31 (*s*, 6H, $H_3C(12^1, 17^1)$), 2.22 (*s*, 3H, $H_3C(8^1)$), 1.93 (*s*, 3H, $H_3C(2^1)$); MS: 610 (M^+). Anal. calcd. for $C_{35}H_{38}N_4O_6$: C, 68.84; H, 6.27; N, 9.17. Found: C, 68.90; H, 6.20; N, 9.30.

Benzyl 3,5-dimethyl-4-acetyl-[^{15}N]-pyrrole-2-carboxylate (**7**) was prepared following the procedure described elsewhere[20] but using [^{15}N]-sodium nitrite (99%, MSD Isotopes); m.p.: 134-136°C; ^{1}H-NMR, δ: 8.97 (*d*, $^3J_{NH}$=98.4, 1H, HN), 2.52 (*d*, $^3J_{NH}$=1.7, 3H, $H_3C(5^1)$); ^{13}C-NMR, d: 195.4 (*d*, $^2J_{CN}$=2.3, $C(4^1)$), 161.4 (*d*, $^2J_{CN}$=2.0, $C(2^1)$), 138.9 (*d*, $^1J_{CN}$=13.4, C(2)), 129.9 (*d*, $^2J_{CN}$=3.6, C(4)), 123.3 (*d*, $^2J_{CN}$=3.6, C(3)), 117.8 (*d*, $^1J_{CN}$=13.4, C(5)), 14.8 (*d*, $^2J_{CN}$=1.5, $H_3C(3^1)$). Anal. calcd. for $C_{16}H_{17}{}^{15}NO_3$: C, 70.59; H, 6.25; N, 5.51. Found: C, 70.62; H, 6.30; N, 5.62.

Benzyl 3,5-dimethyl-4-ethyl-[^{15}N]-pyrrole-2-carboxylate (**8**) was prepared from **7** following the described procedure[11c]; m.p.: 93°C; ^{1}H-NMR, δ: 8.59 (*d*, $^1J_{NH}$=97.6, 1H, HN), 2.40 (*dq*, $^3J_{HH}$=7 and $^4J_{NH}$=1.5, 2H, $H_2C(4^1)$), 2.23 (*d*, $^3J_{NH}$=2.0, 3H, $H_3C(5^1)$), 1.12 (*t*, $^3J_{HH}$=7.0, 3H, $H_3C(4^2)$); ^{13}C-NMR, δ: 161.4 (*d*, $^2J_{CN}$=2.4, $C(2^1)$), 129.6 (*d*, $^1J_{CN}$=14.6, C(2)), 127.2 (*d*, $^2J_{CN}$=4.8, C(4)), 123.8 (*d*, $^2J_{CN}$=4.0, C(3), 116.1 (*d*, $^1J_{CN}$=15.6, C(5)). Anal. calcd. for $C_{16}H_{19}{}^{15}NO_2$: C, 74.42; H, 7.36; N, 5.81.

Benzyl 4-ethyl-3-methyl-5-tert-butyloxycarbonyl-[^{15}N]-pyrrole-2-carboxylate (**9**) was prepared from **8** following the described procedure[21]; m.p.: 88-89°C; ^{13}C-NMR, δ: 160.6 and 160.2 (2x*d*, J_{CN}=2.6 and 2.3, C(2^1, 5^1)), 132.5 and 126.7 (2x*d*, 2xJ_{CN}=3.5, C(3, 4)), 122.7 and 120.8 (2x*d*, 2xJ_{CN}=15.9, C(2, 5)). Anal. calcd. for $C_{20}H_{25}{}^{15}NO_4$: C, 69.77; H, 7.27; N, 4.36. Found: C, 64.90; H, 7.31; N, 4.40.

4-Ethyl-3-methyl-5-tert-butyloxycarbonyl-[^{15}N]-pyrrole-2-carboxylic acid (**10**) was obtained from **9** as described[21]; m.p.: 130-131 °C; ^{13}C-NMR, δ: 160.5 (*d*, $^2J_{CN}$=6.2, C(5^1)), 136.4 (*d*, $^1J_{CN}$=16.2, C(2)), 132.8 (*d*, $^2J_{CN}$=3.4, C(3) or C(4)), 127.9 (*d*, $^2J_{CN}$=5, C(4) or C(3)), 123.4 (*d*, $^1J_{CN}$=15.9, C(5)). Anal. calcd. for $C_{13}H_{19}{}^{15}NO_4$: C, 61.42; H, 7.48; N, 5.90. Found: C, 61.31; H, 7.55; N, 5.98.

tert-Butyl-3-ethyl-5-iodo-4-methyl-[^{15}N]-pyrrole-2-carboxylate (**11**) was obtained from **10** as described[11c]; m.p.: 113°C; ^{1}H-NMR, δ: 8.75 (*d*, $^1J_{NH}$=98.6, 1H, HN), 2.77 (*q*, $^3J_{HH}$=8.0, 2H, $H_2C(3^1)$), 2.01 (*d*, $^4J_{NH}$=1.4, 3H, $H_3C(4^1)$), 1.61 (*s*, 9H, $H_3C(2^4)$), 1.15 (*t*, $^3J_{HH}$=8.0, 3H, $H_3C(3^2)$). Anal. calcd. for $C_{12}H_{18}{}^{15}NO_2I$: C, 42.86; H, 5.36; N, 4.46. Found: 42.92; H, 5.40; N, 4.50.

tert-Butyl-3-ethyl-4-methyl-[^{15}N]-pyrrole-2-carboxylate (**12**) was obtained from **11** as described elsewhere[11c]; m.p.: 103-105°C; ^{1}H-NMR, δ: 8.64 (*dd*, $^1J_{NH}$=98.6 $^2J_{HH}$=3.0, 1H, HN), 6.60 (*t broad*, $^3J_{HH}$=3.0, 1H, HC(5)), 2.73 (*q*, $^3J_{HH}$=8.0, 2H, $H_2C(3^1)$), 2.04 (*d*, $^4J_{NH}$=1.0, 3H, $H_3C(4^1)$), 1.59 (*s*, 9H, $H_3C(2^4)$), 1.15 (*t*, $^3J_{HH}$=8.0, 3H, $H_3C(3^2)$); ^{13}C-NMR, δ: 161,7 (*d*, $^2J_{CN}$=6.3, C(2^1)), 80.1 (C(2^3)), 28.2 (C(2^4)), 18.2 (C(3^1)), 15.1 (C(3^2)), 9.5 (C(4^1)). Anal. calcd. for $C_{12}H_{19}{}^{15}NO_2$: C, 68.57; H, 9.05; N, 7.14. Found: C, 68.63; H, 9.15; N, 7.25.

1-Benzyloxycarbonyl-3-(2-chloroethyl)-2,7-dimethyl-8-ethyl-9-tert-butyloxycarbonyl-[11-^{15}N]-dipyrrylmethane (**14**). A solution of 874 mg of 2-acetoxymethyl-pyrrole **13**[15] in 75 ml of methylene chloride: methanol 7% was slowly added to a solution of 525 mg of [^{15}N]-pyrrole **12** and 20 mg of *p*-toluenesulfonic acid in 30 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 **14** were pooled and evaporated to dryness and gave an oily residue; 1.1 g (85%); MS: 499 (M^+); ^{1}H-NMR, δ: 9.05 (*s*, 1H, HN(10)), 8.71 (*d*, $^1J_{NH}$=98.4, 1H, HN(11)), 7.34 (*s*, 5H, Ph), 5.27 (*s*, 2H, $H_2C(1^3)$), 3.87 (*d*, $^3J_{NH}$=2.0, $H_2C(5)$), 3.44 (*t*, $^3J_{HH}$=7.2, 2H, $H_2C(3^2)$), 2.70 (*m*, 4H, $H_2C(3^1, 8^1)$), 2.30 and 1.98 (2x*s*, 6H, $H_3C(2^1, 7^1)$), 1.55 (*s*, 9H, $H_3C(9^4)$), 1.14 (*t*, $^3J_{HH}$=8.0, 3H, $H_3C(8^2)$). Anal. calcd. for $C_{28}H_{35}{}^{14}N^{15}NO_4Cl$: C, 67.27; H, 7.01; N, 5.80. Found: C, 67.30; H, 6.99; N, 5.91.

1,19-Di-tert-butyloxycarbonyl-13-(2-chloroethyl)-3,7-di-(2-ethoxycarbonylethyl)-18-ethyl-2,8,12,17-tetramethyl-[24-^{15}N]-bilene-b hydrobromide (**17**) was obtained from [^{15}N]-(**14**) (500 mg) followed by the steps of hydrogenolysis to **15** (385 mg, 94%), decarboxylation to **16** (329 mg, 96%), and condensation with **1** (452 mg) as was described above for the synthesis of **4**; 729 mg (87%); m.p.: >300°C (from benzene-hexane). Anal. calcd. for $C_{47}H_{65}{}^{14}N_3{}^{15}NO_8Cl.HBr$: C, 60.68; H, 7.10; N, 6.13. Found: C, 60.70; H, 7.20; N, 6.20.

13-(2-Chloroethyl)-18-ethyl-3,7-di-(2-methoxycarbonylethyl)-2,8,12,17-tetramethyl-[24-^{15}N]-bilin-1,19-dione (**18**) was obtained from [^{15}N]-**17** (400 mg) following the procedure described for **5**; 70 mg (25%); m.p.:

200-201°C; UV-Vis: 642 (4.07), 364 (4.60); ^{1}H-NMR, δ: 6.69 (*s*, 1H, HC(10)), 5.98 (*s*, 1H, HC(5)), 5.84 (*d*, $^3J_{NH}$=4.6, HC(15)), 3.71 and 3.67 (2x*s*, 6H, $H_3C(3^5, 7^5)$), 3.56 (*t*, $^3J_{HH}$=7.6, 2H, $H_2C(13^2)$), 2.95 (*t*, $^3J_{HH}$=7.6, 2H, $H_2C(13^1)$), 2.84 and 2.81 (2x*t*, $^3J_{HH}$=7.7 and 7.6, 4H, $H_2C(3^2, 7^2)$), 2.65 and 2.50 (2x*t*, $^3J_{HH}$=7.7 and 7.6, 4H, $H_2C(3^1, 7^1)$), 2.26 (*q*, $^3J_{HH}$=7.6, 2H, $H_2C(18^1)$), 2.20 and 2.18 (2x*s*, 6H, $H_3C(8^1, 12^1)$), 2.09 (*s*, 3H, $H_3C(17^1)$), 1.84 (*s*, 3H, $H_3C(2^1)$), 1.07 (*t*, $^3J_{HH}$=7.5, 3H, $H_3C(18^2)$); ^{13}C-NMR, δ: 172.9 and 172.7 ($C(3^3, 7^3)$), 172.2 and 171.9 (C(1, 19)), 149.5, 149.3, 143.0, 142.4, 141.8, 141.6, 141.5, 141.0, 140.1, 139.6, 136.0, 135.4, 134.8, 134.3, 130.8, 130.2 and 128.0 (C_{arom}), 114.5 (C(10)), 96.1 and 95.2 (C(5, 15)), 51.5 ($C(3^5, 7^5)$), 43.6 ($C(13^2)$), 32.4 and 33.5 ($C(3^2, 7^2)$), 28.1 ($C(13^1)$), 19.6 ($C(3^1, 7^1)$), 16.7 ($C(18^1)$), 12.5 ($C(18^2)$), 9.3 ($C(8^1, 12^1, 17^1)$), 8.3 ($H_3C(2^1)$). Anal. calcd. for $C_{35}H_{41}{}^{14}N_3{}^{15}NO_6Cl$: C, 64.66; H, 6.36; N, 8.77. Found: C, 64.68; H, 6.35; N, 8,75.

[24-^{15}N]-Dihydroneobiliverdin IXβ dimethyl ester (**19**) was prepared as deep blue prisms from the [24-^{15}N]-bilindione **18** (30 mg) following the procedure described for the obtention of **6**; 16 mg (55%); m.p.: 210-211°C; UV-Vis (pH=6, methanol)[22]: 634 (4.54), 374 (4.66); ^{1}H-NMR, δ: 6.73 (*s*, 1H, HC(10)), 6.25 (*d*, $^3J_{NH}$=5.1, 1H, HC(15)), 6.01 (*s*, 1H, HC(5)), 3.95 (broad *m*, 2H, $H_2C(13^2)$), 3.70 and 3.67 (2x*s*, 6H, $H_3C(3^5, 7^5)$), 2.85 (*m*, 6H, $H_2C(3^2, 7^2, 13^1)$), 2.61 (*t*, $^3J_{HH}$=7.5, 2H, $H_2C(3^1)$); 2.51 (*t*, $^3J_{HH}$=7.5, 2H, $H_2C(7^1)$); 2.39 (*q*, $^3J_{HH}$=7.5, 2H, $H_2C(18^1)$); 2.21 and 2.20 (2x*s*, 9H, $H_3C(8^1, 12^1, 17^1)$), 2.00 (*s*, 3H, $H_3C(2^1)$), 1.11 (*t*, $^3J_{HH}$=7.5, 3H, $H_3C(18^2)$); ^{13}C-NMR, δ: 173.3 and 173.0 ($C(3^3, 7^3)$), 172.4 and 171.2 C(1, 19)), 143.1, 142.9, 142.2, 141.6, 140.1, 139.9, 139.8, 139.6, 138.3, 133.7, 133.5, 133.0, 132.6 and 127.2 (14xC_{arom}), 114.8 (C(10)), 99.1 (C(15)), 97.1 (*d*, $^2J_{CN}$=3.3, C(5)), 51.4 and 51.2 ($H_3C(3^5, 7^5)$), 40.0 (*d*, $^1J_{CN}$=9.8, $C(13^2)$), 34.5 and 33.6 ($C(3^1, 7^1)$), 25.0 ($C(13^1)$), 19.7 ($C(3^2, 7^2)$), 17.2 ($C(18^1)$), 13.0 ($C(18^2)$), 9.3, 9.1, 8.9 and 8.6 ($C(2^1, 8^1, 12^1, 17^1)$). Anal. calcd. for $C_{35}H_{40}{}^{14}N_3{}^{15}NO_6$: C, 68.61; H, 6.58; N, 9.14. Found : C, 68.50; H, 6.55; N, 9.19.

1-tert-Butyloxycarbonyl-8-(2-chloroethyl)-3,7-dimethyl-2-(2-ethoxycarbonylethyl)-9-formyl-dipyrrylmethane (**25**). Dipyrrylmethane **22** (525 mg) was hydrogenated to **23**[23] (413 mg, 93%) and decarboxylated to **24** (319 mg, 85%) according to the procedure described for dipyrrylmethane **3**. A solution of 319 mg of dipyrrylmethane **24** in 0.4 ml of dimethylformamide was kept at 5°C and 0.4 ml of benzoyl chloride was 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; 237 mg (70%); ^{1}H-NMR, δ: 9.5 (*s*, 1H, $HC(9^1)$), 4.19 (*q*, $^3J_{HH}$=7.2, 2H, $H_2C(2^5)$), 4.00 (*s*, 2H, $H_2C(5)$), 3.55 (*t*, $^3J_{HH}$=6.1, 2H, $H_2C(8^2)$), 3.05-2.45 (*m*, 6H, $H_2C(2^1, 2^2, 8^1)$), 2.33 and 2.26 (2x*s*, 6H, $H_3C(3^1, 7^1)$), 1.56 (*s*, 9H, $H_3C(1^4)$), 1.27 (*t*, $^3J_{HH}$=7.2, 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, 7.23; N, 6.15.

1-Benzyloxycarbonyl-9-tert-butyloxycarbonyl-8-(2-ethoxycarbonylethyl)-3-ethyl-2,7-dimethyl-[10-^{15}N]-dipyrrylmethane (**26**) was prepared as described[24] by condensation of the 2-acetoxymethyl-[^{15}N]-pyrrole **20** (prepared as described for the [^{14}N]-isotopomer[11c]) and the 2-*tert*-butyloxycarbonyl pyrrole **21**[25]; ^{1}H-NMR, δ: 8.95 (*d*, $^1J_{NH}$=92.0, 1H, HN), 8.85 (*s*, 1H, HN), 7.39 (*s*, 5H, Ph), 5.26 (*s*, 2H, $H_2C(1^3)$), 4.15 (*q*, $^3J_{HH}$=7.0, 2H, $H_2C(8^5)$), 3.82 (*s*, 2H, $H_2C(5)$), 3.15-2.35 (*m*, 6H, $H_2C(3^1, 8^1, 8^2)$), 2.31 and 2.01 (2x*s*,

6H, $H_3C(2^1, 7^1)$, 1.60 (*s*, 9H, $H_3C(9^4)$), 1.30 (*t*, $^3J_{HH}$=7.2, 3H, $H_3C(8^6)$), 1.08 (*t*, $^3J_{HH}$=7.0, 6H, $H_3C(3^2)$). Anal. calcd. for $C_{31}H_{39}{}^{14}N^{15}NO_6$: C, 69.40; H, 7.28; N, 5.41. Found: C, 69.35; H, 7.20; N, 5.56.

1,19-Di-tert-butyloxycarbonyl-8-(2-chloroethyl)-2,18-di-(2-ethoxycarbonylethyl)-13-ethyl-3,7,12,17-tetramethyl-[23-^{15}N]-bilene-b hydrobromide (**29**) was prepared following the procedure described for **4**. [^{15}N]-Dipyrrylmethane **26** (380 mg) was transformed into [^{15}N]-dipyrrylmethane **28** (205 mg, 72%) by hydrogenolysis and decarboxylation, and the latter was condensed with formyl dipyrrylmethane **25** (237 mg) as described; 403 mg (85%) of hydrobromide **29** were obtained; m.p.: above 300°C. Anal. calcd. for $C_{47}H_{65}{}^{14}N_3{}^{15}NO_8Cl.HBr$: C, 60.62; H, 7.09; N, 6.12. Found : C, 60.68; H, 7.11; N, 6.10.

8-(2-Chloroethyl)-2,18-di-(2-methoxycarbonylethyl)-13-ethyl-3,7,12,17-tetramethyl-[23-^{15}N]-bilin-1,19-dione (**30**) was prepared from [^{15}N]-bilene-b **29** (280 mg) following the procedure described for **5**, 68 mg (35%) of deep blue prisms were obtained; m.p.: 202-204°C (benzene-heptane); UV-Vis: 631 (4.20), 369 (4.74); ^{1}H-NMR, δ: 6.52 (*d*, $^3J_{NH}$=4.6, 1H, HC(10)), 5.80 (*s*, 1H, HC(5)), 5.79 (*d*, $^3J_{NH}$=4.2, 1H, HC(15)), 4.21 (*dq*, $^3J_{HH}$=7.5 and $^3J_{NH}$=5.9, 2H, $H_2C(13^1)$), 3.65 (*s*, 6H, $H_3C(2^5, 18^5)$), 3.57 (*t*, $^3J_{HH}$=7.6, 2H, $H_2C(8^2)$), 3.00 (*t*, $^3J_{HH}$=7.6, 2H, $H_2C(8^1)$), 2.54 (*s*, 8H, $H_2C(2^1, 2^2, 18^1, 18^2)$), 2.13, 2.09, 2.08 and 2.05 (4x*s*, 12H, $H_3C(3^1, 7^1, 12^1, 17^1)$), 1.09 (*t*, $^3J_{HH}$=7.5, 3H, $H_3C(13^2)$). MS.: 649 M^+. Anal. calcd. for $C_{35}H_{41}{}^{14}N_3{}^{15}NO_6Cl$: C, 64.66; H, 6.36; N, 8.77. Found: C, 64.68; H, 6.35; N, 8.75.

[23-^{15}N]-Dihydrophorcabilin dimethyl ester (**31**) was obtained from **30** (68 mg) following the procedure described for the synthesis of neobiliverdin IXβ **6**; blue violet prisms; 49 mg (76%); m.p.: dec. above 150°C; UV-Vis: 597 (4.51), 391 (4.21); ^{1}H-NMR, δ: 6.86 (*d*, $^3J_{NH}$=5.0, 1H, HC(10)), 5.83 (*d* ,$^3J_{NH}$=1.7, 1H, HC(5)), 5.80 (*s*, 1H, HC(15)), 3.90 (*m*, 2H, $H_2C(8^2)$), 3.66 and 3.61 (2x*s*, 6H, $H_3C(2^5, 18^5)$), 2.90(*m*, 2H, $H_2C(8^1)$), 2.62 (*m*, 8H, $H_2C(2^1, 2^2, 18^1, 18^2)$), 2.32 (*q*, $^3J_{HH}$=7.5, 2H, $H_2C(13^1)$), 2.15, 2.12, 2.06 and 1.81 (4x*s*, 12H, $H_3C(3^1, 7^1, 12^1, 17^1)$), 0.97 (*t*, $^3J_{HH}$=7.5, 3H, $H_3C(13^2)$); ^{13}C-NMR, δ: 173.6 ($C(2^3, 18^3)$), 173.0 (C(1, 19)), 171.0, 163.5, 147.1, 142.4, 141.5, 140.7, 138.8, 134.6, 133.3, 133.2, 132.5, 131.5, 128.3 and 128.2 (C_{arom}), 118.2 (C(10)), 96.4 and 92.3 (C(5, 15)), 51.4 and 51.2 ($C(2^5, 18^5)$), 45.6 (*d*, $^1J_{CN}$= 10.2, ($C(8^2)$), 31.7 ($C(2^2, 18^2)$), 28.8 ($C(8^1)$), 22.5 ($C(2^1, 18^1)$), 19.8 ($C(13^1)$), 14.7 ($C(13^2)$), 9.5 and 8.9 ($C(3^1, 7^1, 12^1, 17^1)$). Anal. calcd. for $C_{35}H_{40}{}^{14}N_3{}^{15}NO_6$: C, 68.51; H, 6.52; N, 9.30. Found : C, 68.55; H, 6.50; N, 9.35.

1,19-Di-tert-butyloxycarbonyl-12,17-di-(2-chloroethyl)-3,7-di-(2-ethoxycarbonylethyl)-2,8,13,18-tetramethyl-bilene-b hydrobromide (**35**) was obtained following the procedure described for **4**. Dipyrrylmethane **32**[26] (180 mg) was reduced with hydrogen to the acid **33** (145 mg, 97%), which was decarboxylated with *p*-toluenesulfonic acid in methylene chloride-methanol to give the dipyrrylmethane **34** (120 mg, 92%). The latter was then condensed with the dipyrrylmethane **1** (151 mg) to give the bilene-b hydrobromide **35** as a bright brilliant red crystalline solid; 266 mg (96%); m.p.: above 300°C; ^{1}H-NMR, δ: 13.30, 10.20, 9.90 (3x*s broad*, 4H, HN), 4.50-3.95 (*m*, 8H, $H_2C(3^5, 5, 7^5, 15)$), 3.75-3.15 (*m*, 16H, $H_2C(3^1, 3^2, 7^1, 7^2, 12^1, 12^2, 17^1, 17^2)$), 2.28, 2.25 and 2.07 (3x*s*, 12H, $H_3C(2^1, 8^1, 13^1, 18^1)$), 1.60 (*s*, 18H, $H_3C(1^4, 19^4)$), 1.25 (*t*, $^3J_{HH}$=7.5, 6H, $H_3C(3^6, 7^6)$). Anal. calcd. for $C_{47}H_{64}Cl_2N_4O_8.HBr$: C, 58.51; H, 6.79; N, 5.81. Found: C, 58.55; H, 6.80; N, 5.80.

3,7-Di-(2-methoxycarbonylethyl)-2,8,13,18-tetramethyl-12,17-di-(2-chloroethyl)-bilin-1,19-dione (**36**) was obtained from **35** following the procedure described for **5**. From 250 mg of bilene-b **35** were obtained 51

mg (29%) of turquoise-blue prisms; m.p.: 200-201°C (from benzene-hexane); UV-Vis: 647 (4.11), 364 (4.65); ^{1}H-NMR, δ: 6.70 (*s*, 1H, C(10)), 6.03 and 5.96 (2x*s*, 2H, C(5, 15)), 3.71 and 3.67 (2x*s*, 6H, $H_3C(3^5, 7^5)$), 3.61 (*t*, $^3J_{HH}$=7.5, 4H, $H_2C(12^2, 17^2)$), 3.00 (*m*, 4H, $H_2C(12^1, 17^1)$), 2.84 (*m*, 4H, $H_2C(3^1, 7^1)$), 2.65 (*t*, $^3J_{HH}$=7.5, 2H, $H_2C(3^2)$), 2.51 (*t*, $^3J_{HH}$=7.5, 2H, $H_2C(7^2)$), 2.25 and 2.15 (2x*s*, 6H, $H_3C(8^1, 13^1)$), 1.85 (*s*, 6H, $H_3C(2^1, 19^1)$). Anal. calcd. for $C_{35}H_{40}Cl_2N_4O_6$: C, 61.49; H, 5.90; N, 8.20. Found: C, 61.51; H, 5.70; N, 8.25.

Neobiliverdin IXδ (**37**) was obtained from **36** following the procedure described for the obtention of neobiliverdin IXβ **6**; from 48 mg of **36** were obtained 26 mg (61%) of **37** as blue-violet prisms; m.p.: 108-110°C.; UV-Vis: 598 (4.27), 393 (3.34). Anal. calcd. for $C_{35}H_{38}N_4O_6$: C, 68.84; H, 6.27; N, 9.17. Found: C, 68.80; H, 6.30; N, 9.25. NMR data are coincident with those obtained by Benedikt, E. et al.[16].

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