# Synthesis and Stereochemistry of a Helical 2,18-Bridged Biliverdin

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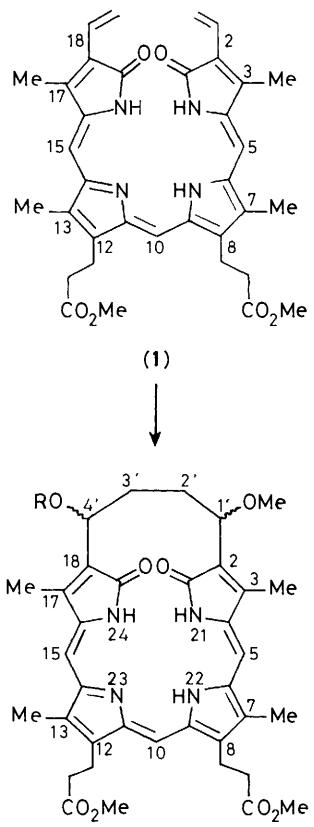
Biliverdin-IIIα dimethyl ester (**1**) when treated with acidic methanol undergoes ring closure of the exocyclic vinyl groups with consecutive addition of two methoxy groups and oxidation, predominantly affording (**3**). The four-membered bridge connecting the pyrrolinone rings possesses two chiral centres of equal configuration either (1′*R*,4′*R*) or (1′*S*,4′*S*). Due to the *M*/*P* helicity of the bilatriene backbone, (**3**) comprises two thermally interconvertible diastereoisomeric pairs of enantiomers, *viz.* (**3a**) of configuration [(*P*,1′*R*,4′*R*) and (*M*,1′*S*,4′*S*)] and (**3b**) of configuration [(*M*,1′*R*,4′*R*) and (*P*,1′*S*,4′*S*)]. Their stereochemistry was assigned by n.m.r. techniques. The flexibility of the bilatriene unit is restricted so the separation of (**3a**) and (**3b**) can be seen at moderately low temperatures. Despite geometric contraints the torsional angles of the bilatriene backbones are close to non-bridged helical shaped derivatives. A mechanism for the acid mediated cyclisation reaction is proposed and briefly discussed.

Biliverdins are used as model compounds for the photoreceptor phytochrome, a chromoprotein responsible for photomorphogenesis in higher plants.[1] *In vivo* this biliprotein exists in two forms which are photochemically interconvertible. On denaturation of the apoprotein a transition from a stretched to a helical conformation of the tetrapyrrolic prosthetic group occurs. This assumption mostly rests upon a prediction of theory[2] which states that the absorption quotient of the first u.v.- and the visible-electronic absorption band of a given bilatriene decreases if its geometry is changed from a helical to a more stretched conformation. Few compounds with fixed geometry have hitherto been synthesized[3] to confirm the general validity of this criterion. The difficulties one is faced with if two bilatrienes are compared arise from additional factors prone to modify electronic absorption spectra *e.g.* substitution pattern and solvation.

In the course of our investigations into the influence of covalently bound peptides on the conformationally labile bilatriene backbone[4] we needed compounds with fixed helical geometry. If geometry is fixed by a methylene bridge inserted between N-21 and N-24 large deviations from the initial pitch of the helix occur,[5] thus preventing any meaningful comparison with non-bridged compounds. This prompts us to report our results on the synthesis of appropriate biliverdins.

## Results and Discussion

If (**1**) is treated with oxygen free methanolic hydrochloric acid (0.8M) at room temperature, traces (<5%) of a second compound (**2**) appear after 1 h (by t.l.c.). If reaction time is prolonged the amount of (**2**) at first increases but then decreases again at the expense of an additional compound (**3**). After 48 h only traces of both (**1**) and the intermediate (**2**) remain and the yield of the final product (**3**) amounts to *ca.* 30%. This reaction only proceeds with satisfactory rate and yield if: (i), strictly anhydrous conditions are avoided and (ii), the initial concentration of (**1**) in acidic methanol is not as low as $10^{-4}$M. Otherwise a greater or lesser variety of unidentified by-products is obtained. Thus the above reaction is best performed with solutions $10^{-2}$M in (**1**) containing water in 0.1—0.5% (v/v). Lowering the amount of acid likewise retards reaction rate but leads to an increased formation of intermediate (**2**) up to a certain time of reaction; additionally the formation of by-products is suppressed. In contrast the isomers of (**1**), namely




| R = H | R = Me | |
|---|---|---|
| (**2a**) | (**3a**) | [(*P*,1′*R*,4′*R*) + (*M*,1′*S*,4′*S*)] |
| (**2b**) | (**3b**) | [(*M*,1′*R*,4′*R*) + (*P*,1′*S*,4′*S*)] |
| | (**4**) | [(*P*,1′*R*,4′*S*) + (*M*,1′*S*,4′*R*)] |

**Scheme 1.**

biliverdin-IXα-and XIIIα-dimethyl ester in which the vinyl groups are located at positions 3 and 18, and 3 and 17, respectively, exhibit a remarkable stability towards acidic methanol under these conditions. Even after 3 days neither corresponding reactions nor decomposition products are

**Table 1.** Isomeric distribution (%) as determined by $^1$H n.m.r. of equilibrated mixtures[a] of (**2a**)/(**2b**) and (**3a**)/(**3b**) in various solvents at *ca.* 5 × $10^{-3}$M at 293 K[b]

| | (2) | | (3) | |
|---|---|---|---|---|
| | (a) | (b) | (a) | (b) |
| $CH_2Cl_2$ | | *c* | 80 | 20 |
| EtOH | 74 | 26 | 73 | 27 |
| $CHCl_3$ | >95 | <5 | 72 | 28 |
| DMSO | | *c* | 71 | 29 |
| 1,4-Dioxane | 67 | 33 | 46 | 54 |
| $C_6H_6$ | 85 | 15 | 30 | 70 |

[a] Equilibrium is attained after *ca.* 30 min. [b] Error ±2%. [c] Not performed.

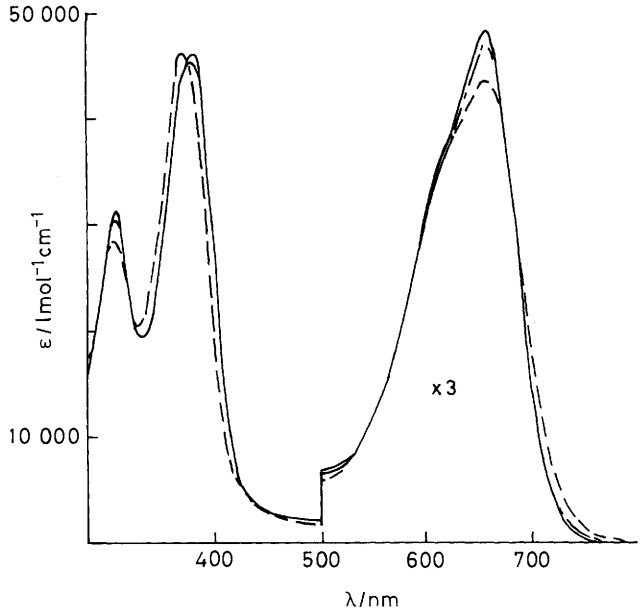



**Figure 1.** U.v.–visible electronic absorption spectra of diastereoisomers (**3a**) (——) and (**3b**) (– – –) and of the equilibrated mixture (— – —) for 5 × $10^{-5}$M solutions in ethanol at 278 K

observed. The occurrence of the new compound (**3**) and its intermediate (**2**) formed from (**1**) may thus plausibly be associated with the substitution pattern. The vinyl groups located at positions 2 and 18 of (**1**) are in close proximity if a helical conformation is adopted.

If t.l.c. of the final product (**3**) is carried out at lower temperatures (< 0 °C) it turns out to be no longer homogeneous but two compounds (**3a**) and (**3b**) (with half lives of *ca.* 3 min at 20 °C) appear. In fact, in the $^1$H n.m.r. even at ambient temperature two thermally interconvertible species (**3a**) and (**3b**) equal in their $C_2$-symmetry can be seen, their isomer ratio being particularly sensitive towards the solvent employed (Table 1). For both compounds a four membered bridge formed from the vinyl groups of (**1**) is characteristic. It connects the pyrrolinone rings resulting in a covalently fixed cyclic structure. Additionally, two methoxy groups are located at the respective 1′- and 4′-positions of the bridge. The structural and stereochemical assignments as outlined in Scheme 1 rest upon $^1$H n.m.r. investigations (deuterium exchange, decoupling experiments, and n.O.e. difference spectra), $^{13}$C n.m.r. spectra, field desorption mass spectrometry, and u.v.–visible absorption spectra. Both compounds (**3a**) and (**3b**) exhibit n.O.e.s of the C-7 (C-13) methyl group and of the C-3 (C-17) methyl group with the meso proton attached to C-5 (C-15) and of the methoxy group at the C-1′ (C-4′) position of the bridge to the corresponding methine proton at the same position. However species (**3a**) and (**3b**) differ in the spatial relationships of the respective C-3 (C-17) methyl group to both the methine proton and methoxy group in position C-1′ (C-4′) of the bridge: species (**3a**) exhibits an additional n.O.e. of the C-3 (C-17) methyl group to the methine proton at C-1′ (C-4′). On the other hand for species (**3b**) a n.O.e. of the methoxy group at C-1′ (C-4′) to the methyl group at C-3 (C-17) and *vice versa* is characteristic. Coupling between the methine proton at the C-1′ (C-4′) position with the C-2′ (C-3′) methylene protons occur both in (**3a**) and (**3b**). For (**3b**) an additional long range coupling of the methine proton with the methyl group at C-3 (C-17) of the bilatriene unit is observed.

The distinction between species (**3a**) and (**3b**) and the reason for their thermal interconvertibility may be understood if all elements of chirality are taken into account. In the course of the reaction two chiral centres in the respective C-1′ and C-4′ positions of (**3a**) and (**3b**) are formed. A third source of chirality arises from the helicity of the bilatriene backbone. Since both isomers have a $C_2$-axis, as follows from their n.m.r. spectra, the chiral centres in the bridges must always belong to the same configuration *i.e.* either (1′*R*,4′*R*) *or* (1′*S*,4′*S*). Thus the occurrence of species (**3a**) and (**3b**) may plausibly be expressed in terms of chiral diastereoisomers of configuration [(*P*,1′*R*,4′*R*) + (*M*,1′*S*,4′*S*)] and [(*M*,1*R*,4′*R*) + (*P*,1′*S*,4′*S*)] or *vice versa* (for individual assignments see below). Their mutual thermal interconversion is caused by inversion of the kinetically labile bilatriene helix. However, since both species can be seen in the n.m.r. spectra at room temperature and even up to 130 °C no coalescence phenomena occur the barrier to helix inversion must be strongly enhanced when compared with non-bridged bilatrienes for which interconversion is rapid on the n.m.r. time scale at ambient temperature.[6,7]

Typical, for the fixed geometry of (**3**), are the small changes in absorption spectra that occur if conditions are met which in open chain bilatrienes lead to dramatic changes of absorption spectra and which have been associated with conformational changes, such as, protonation,[8] or dissolution in hexamethylenephosphoric acid triamide (HMPA).[9] In Table 2 the behaviour of (**3**) and biliverdin-XIIIα dimethyl ester are compared. Since (**3**) cannot adopt an extended non-helical conformation no substantial spectral changes occur under these conditions thus contrasting the remarkable changes observed for biliverdin-XIIIα dimethyl ester. However, the insensitivity of the u.v.–visible spectrum of (**3**) in benzene towards addition of trifluoroacetic acid in excess might alternatively be explained in terms of hindrance of protonation of the conformationally fixed bilatriene backbone. Accordingly, at present we cannot decide whether protonation occurs at all under these conditions. Nevertheless, both explanations rest upon the rigid cyclic structure of (**3**). The same arguments hold true for the precursor (**2**) (see below).

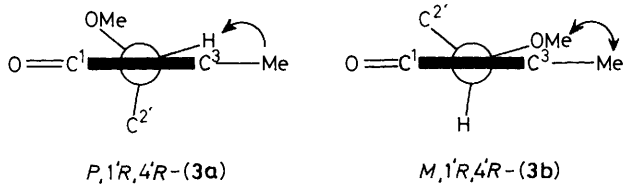
The geometry, *i.e.* torsional angles and pitch of the bilatriene helices of isomers (**3a**) and (**3b**) are very similar. This conclusion can be drawn from the minute differences of the u.v.–visible spectra (Figure 1). In addition the ratio of extinction coefficients of the u.v. and visible band ($\varepsilon^{u.v.}_{max.}/\varepsilon^{visible}_{max.}$) for ethanol solutions amounts to f = 2.9 and f = 3.2 for (**3a**) and (**3b**), respectively, close to helical shaped non-bridged biliverdins, *e.g.* biliverdin-XIIIα dimethyl ester (f = 2.9) void of hyperchromic groups such as vinyl at the 2 and/or 18 position. Since f has been shown to be particularly sensitive towards conformational changes by this criterion it can be concluded that the four membered bridges of (**3a**) and (**3b**) do not exert appreciable strain upon the bilatriene moiety but only impede helix inversion.

Based on the similarities in geometry the stereochemistry of (**3a**) and (**3b**) can be elucidated. In essence the spatial relations

**Table 2.** U.v.–visible absorption spectra $\varepsilon_{max.}$/l mol$^{-1}$ cm$^{-1}$ ($\lambda_{max.}$/nm) of biliverdin-XIIIα dimethyl ester (BVE-XIIIα) and of equilibrated[a] mixtures of (**2a**)/(**2b**) and (**3a**)/(**3b**) in various solvents at *ca.* 5 × 10$^{-5}$M at 293 K. The factors [f] refer to the respective quotients of extinction coefficients of the u.v.- and visible absorption bands

| | BVE-XIIIα | (**2a**)/(**2b**) | (**3a**)/(**3b**) |
|---|---|---|---|
| $CHCl_3$ | 15 300 (651)<br>44 300 (377) [2.9] | 15 200 (658)<br>45 600 (384) [3.0] | 15 300 (660)<br>46 600 (384) [3.0] |
| $CH_2Cl_2$ | 15 400 (653)<br>44 400 (377) [2.9] | 15 200 (656)<br>46 000 (384) [3.0] | 15 300 (658)<br>46 000 (384) [3.0] |
| EtOH | 15 300 (656)<br>44 700 (376) [2.9] | 15 200 (659)<br>45 400 (380) [3.0] | 15 200 (661)<br>45 500 (380) [3.0] |
| THF | 16 500 (642)<br>43 700 (376) [2.6] | 16 000 (657)<br>45 900 (379) [2.9] | 15 200 (661)<br>44 300 (378) [2.9] |
| $C_6H_6$ | 16 300 (651)<br>43 200 (380) [2.6] | 16 200 (656)<br>43 500 (384) [2.7] | 15 700 (658)<br>43 400 (384) [2.8] |
| $C_6H_6/H^+$[b] | 33 900 (662)<br>47 400 (378) [1.4] | 14 800 (652)<br>40 200 (385) [2.7] | 15 200 (655)<br>40 600 (384) [2.7] |
| HMPA | 26 500 (647)<br>38 500 (383) [1.5] | 15 700 (668)<br>44 300 (378) [2.8] | 16 300 (673)<br>43 900 (384) [2.7] |

[a] See footnote *a* to Table 1. [b] Addition of 10 equiv. trifluoroacetic acid.




*P*,1′*R*,4′*R*-(**3a**) *M*,1′*R*,4′*R*-(**3b**)

**Figure 2.** Projection in the plane of the pyrrolinone ring along the C(2)–C(1′)-bond for (**3a**) and (**3b**). The same relations are obtained along the C(18)–C(4′)-bond. Only one configuration displayed. Arrows indicate n.O.e.s on which stereochemical assignment is based

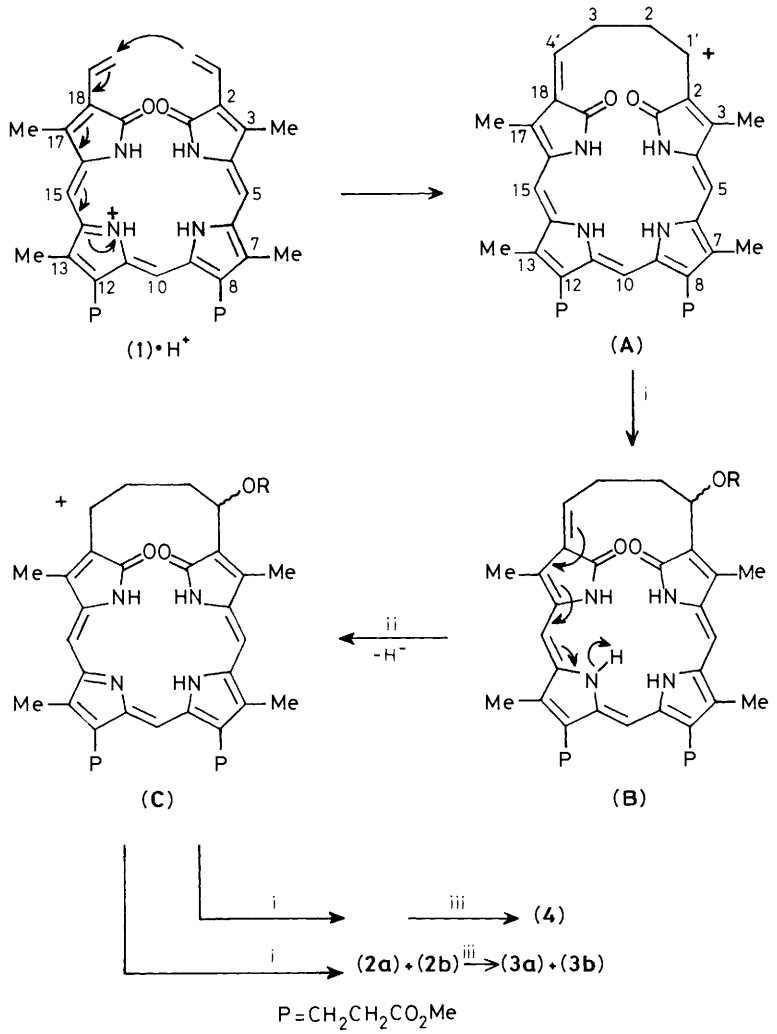
drawn in Figure 2 hold true irrespective of the torsional angles $\varphi_{1',2'}$ ($\varphi_{4',3'}$) and $\varphi_{2',3'}$ as long as the geometry of the helix is preserved. The most prominent differences are the proximity of the respective C-3 (C-17) methyl group to the proton attached to the chirality centre at C-1′ (C-4′) in (**3a**) and to the methoxy group in (**3b**). This reflects the distinctions obtained from n.O.e.s and leads to the stereochemical assignment as outlined in Scheme 1.

Since in addition to (**3a**) and (**3b**) a third diastereoisomer, namely (**4**), should exist the question arose whether this compound has not formed at all or escaped isolation. In fact a closer inspection of some minor reaction products revealed compound (**4**) to be present in amounts less than 5% of that of the major isomers (**3**). Like (**3a**) and (**3b**), (**4**) is chiral. However, while helix inversion in (**3a**) and (**3b**) is associated with mutual transformation of diastereoisomers, in the case of (**4**) this process is equivalent to the interconversion of enantiomers. This is due to the difference in configuration of the chiral centres. Compound (**4**) shows all the characteristics of (**3**) and in essence behaves like an equimolar mixture of (**3a**) and (**3b**). Unfortunately because of the small amounts available no extensive studies have been performed.

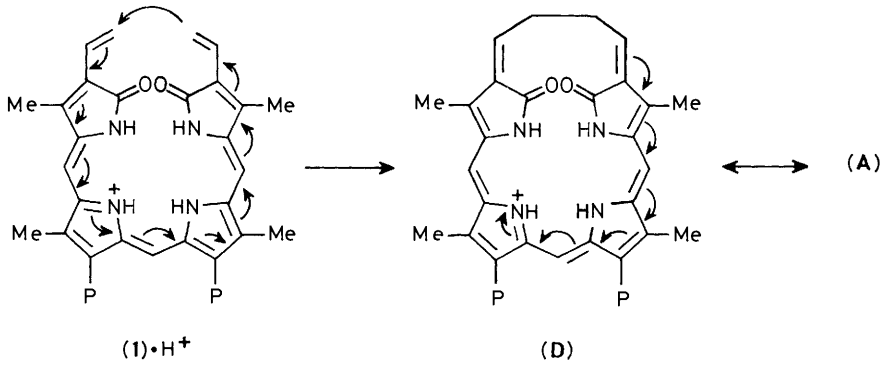
The trapped intermediate (**2**) likewise turns out to be a diastereoisomeric mixture of two bridged biliverdins. With exception of symmetry ($C_1$), (**2a**) and (**2b**) show great similarities with isomers (**3a**) and (**3b**) with respect to both $^1$H n.m.r. and u.v.–visible spectra (Table 2) and were identified as monohydroxy derivatives. Their stereochemistry was assigned by comparison with (**3a**) and (**3b**). The two intermediate carbinols, the probable precursors of the dimethoxy compound (**4**) escaped isolation in our hands.

The small but distinct solvatochromism observed for (**2**) and (**3**) in benzene, halocarbons, ethanol, and tetrahydrofuran essentially does not differ from that observed for biliverdin-XIIIα dimethyl ester (Table 2). The minute variations can plausibly be attributed to solvent dependence of individual spectra of components (**a**) and (**b**) of (**2**) and (**3**) rather than to a change of population. This is in accord with the observation that for (**2**) and (**3**) the f-values are very similar if the u.v.–visible spectra in the same solvent are compared though populations may differ appreciably (Table 1).

The structures given for compounds (**2**), (**3**), and (**4**) imply that their formation from (**1**) cannot simply be explained in terms of intramolecular cyclisation *via* vinyl groups and addition of methanol and water but must necessarily also include an oxidation step. Thus to put forward a mechanism the following facts have to be considered: (i) the methoxy- or hydroxy-groups regioselectively enter into positions 1′ and 4′ of the bridge; (ii) the process is stereoselective and (iii) catalysed by small amounts of water; (iv) at low initial concentrations of (**1**) the reaction rate is strongly retarded; (v) if the reaction is carried out in methan[$^2$H]ol–deuteriotrifluoroacetic acid no deuterium is introduced into the bridges of (**2**) and (**3**), nor in any other C-position. The latter finding excludes direct addition of methanol or water to one or both vinyl groups of (**1**) followed by oxidation. Moreover, it excludes any mechanism in which a $\Delta_{1',2'}$-ene or an $\Delta_{1',2'}$-$\Delta_{3',4'}$-diene might serve as bridged intermediate. The catalysing effect of water and the occurrence of intermediate (**2**) simply implies that in one rate determining step water reacts more readily than methanol. The pathway proposed (Scheme 2) fits all these experimental requirements. Reaction is initiated by the protonated species (**1**)·H$^+$; the exceptional reactivity of the vinyl groups at positions 2 and 18 may be understood in terms of vinylogy. Accordingly the positive charge located at the pyrrolenine nitrogen can be transmitted to one of the vinyl groups *via* π-electrons (Scheme 2). Hence one vinyl group in (**1**)·H$^+$ may be regarded as a vinylogous cation. This also accounts for the formation of a four-membered bridge. Due to the proximity of the second vinyl group the potential vinyl cation (**1**)·H$^+$ undergoes electrophilic reaction with the double bond, namely with the site of higher electron density. Thus a helical conformation for (**1**)·H$^+$ in the transition state is a prerequisite for intramolecular cyclisation although this conformation in the ground state is probably populated only to a small extent. If displacement of double bonds in (**1**)·H$^+$ during ring closure is extended to the *whole* bilatriene system a mesomer of intermediate (**A**), namely (**D**) is formed in which the positive charge is still located at nitrogen (Scheme 3). As has been suggested by a referee this step would represent a 20π




**Scheme 2.** *Reagents:* i, ROH (R = H or Me); ii, oxidant (**1**)•$H^+$; iii, MeOH/$H^+$




**Scheme 3.**

thermally allowed electrocyclic reaction which might also proceed in the absence of acid. Until now attempts to cyclise neutral (**1**) failed.

The ring closed cationic intermediate (**A**) thus formed is then subject to reaction with methanol or water affording (**B**) which subsequently is capable of oxidation to yield (**C**) with in all likelihood the protonated ester (**1**)•$H^+$ itself serving as the oxidizing agent. This would account for the considerable concentration dependence of the reaction. This assumption is corroborated by additional experimental work on cross-experiments with (**1**) and its IXα-isomer. Figure 3 shows that reaction is accelerated if the initial concentration of (**1**) is increased as already stated above (runs 1 and 2). However, rate also increases if biliverdin-IXα dimethyl ester is added to solutions of (**1**) (runs 3 and 4). Apparently, although the IXα-isomer cannot cyclize because of its substitution pattern it may serve as an appropriate oxidant. Even if the concentration of (**1**) in runs 1 and 3 differs by a factor five the reaction rates are equal if the smaller concentration is compensated by addition of the IXα-isomer. Other potential oxidizing agents (DDQ, oxygen, acetaldehyde) used did not promote the formation of bridged compounds but occasionally led to increasing amounts

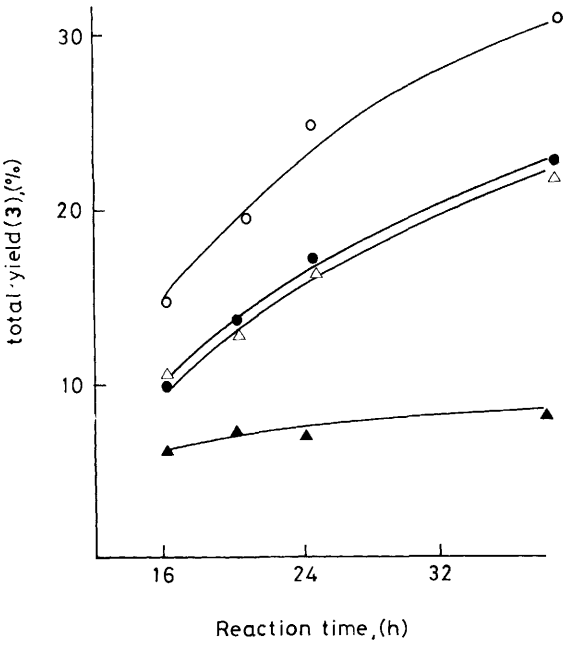



**Figure 3.** Total yield of (**3**) *vs.* reaction time and initial concentration of (**1**) (●, run 1, $c = 1 \times 10^{-2}$M; ▲, run 2, $c = 2 \times 10^{-3}$M) and promotion of reaction on addition of biliverdin-IXα dimethyl ester (BVE-IXα) [△, run 3: (**1**) $c = 2 \times 10^{-3}$M, (BVE-IXα) $c = 8 \times 10^{-3}$M; ○, run 4: (**1**) $c = 1 \times 10^{-2}$M, (BVE-IXα) $c = 4 \times 10^{-2}$M]

of degradation products. Unfortunately tetrapyrrolic reduction products could not be trapped, either because of their instability under the conditions employed or because the site of (**1**)·$H^+$ or its IXα-analogon to which a hydride ion* is transferred is unspecific.

Hence if concentration of biliverdin esters is low *a priori* the reaction step (**B**) ⟶ (**C**) becomes unlikely and (**B**) undergoes irreversible side reactions leading to a spectrum of by-products. Species (**C**) again suffers attack from methanol or water giving the isomeric hydroxy compounds (**2a**) and (**2b**) which finally are methylated affording (**3a**) and (**3b**).

One might argue that formation of the macrocycles proceeds *via* a bilirubin like intermediate formed from (**B**) by addition of ROH prior to oxidation. However, since no deuterium is found at the C-10 methine position of (**2**) and (**3**) if deuteriated reagents are used this alternative route can be excluded.

The stereoselectivity of the reaction observed most plausibly is a consequence of the different steric requirements of the four stereoisomeric species of intermediate (**C**) on addition of ROH. Apparently, activation energy is lower if ROH enters from that side resulting in chiral centres of equal configuration.

If (**4**) or a mixture of (**3a**) and (**3b**) is subjected to the reaction conditions no equilibration (**4**) ⇌ (**3a**) + (**3b**) takes place within 24 h. This is remarkable in view of the additional finding that thereby the methoxy methyl groups at positions C-1′ and C-4′ are replaced by trideuteriomethyl groups if [$^2H_4$]methanol is used as a reagent. Obviously solvolysis of the pair [(**3a**) + (**3b**)] and isomer (**4**) occurs with retention of configuration and does not proceed *via* a common intermediate such as (**C**).

In conclusion we have illustrated the versatility of biliverdin-IIIα dimethyl ester (**1**) as precursor for conformationally fixed, unstrained bilatrienes. These compounds will be useful in our investigations on the conformational changes of natural bilatrienes if covalently bound to peptides. We have further shown that bridging causes inversion of the bilatriene helix to be hindered. Compounds (**2**), (**3**), and (**4**) therefore are promising candidates for optical resolution of the bilatriene helices at moderate low temperatures. Efforts in this direction are presently underway.†

* This notation comprises a formalism and does not exclude a stepwise oxidation of intermediate (**B**) by electron transfer to (**1**)·$H^+$.

† *C.f.* D. Krois and H. Lehner, *J. Chem. Soc., Perkin Trans. 2*, 1989, 2085.

## Experimental

M.p.s were determined with a Kofler-Reichert hot-stage apparatus. $^1H$ N.m.r. (250 MHz) and $^{13}C$ n.m.r. (62.9 MHz; J-modulated) spectra were recorded with a Bruker WM 250 instrument for *ca.* $10^{-2}$M solutions in $CDCl_3$, $CD_2Cl_2$, [$^2H_6$]benzene, [$^2H_6$]ethanol, [$^2H_8$]dioxane, and [$^2H_6$]DMSO using $SiMe_4$ as an internal reference; n.O.e. difference spectra were obtained for $2 \times 10^{-3}$M deaerated solutions. Field desorption mass spectrometry was performed with a Finnegan MAT 312 instrument. U.v.–visible spectra were measured with a Perkin–Elmer Lambda 7 spectrometer in thermostatted quartz cuvettes (0.1—1 cm path length) using spectroscopic grade (Uvasol, Merck) benzene, dichloromethane, tetrahydrofuran (distilled from $LiAlH_4$ prior to use), ethanol, and chloroform (chromatographed on alumina prior to use). For quantitative evaluation of t.l.c. chromatograms a densitometer Karl Zeiss ZK 3 (source slit modified) at λ 630 nm was used. T.l.c. was performed with Kieselgel 60 plates (Merck) in thermostatted trays. For column chromatography Kieselgel 60 (230—400 mesh, Merck) was used. As eluants, p.A. grade (Fluka or Loba) dichloromethane, acetone, toluene, propan-2-ol, and ethyl acetate were employed. All reactions were carried out under argon and protected from light. Anhydrous methanol (distilled from Mg) was deaerated by three freeze-pump-thaw cycles. HMPA (Merck) was distilled *in vacuo*.

*Biliverdin-IIIα Dimethyl Ester* (**1**).—Biliverdin-IIIα (58 mg, 0.1 mmol) obtained from bilirubin-IIIα by oxidation with DDQ[10] was dissolved at 0 °C in methanol–sulphuric acid (20:1) (10 ml). After 1 h at 5 °C the reaction was quenched with aqueous sodium hydrogen carbonate and extracted with $CH_2Cl_2$. The extract was washed with water, dried ($Na_2SO_4$), and the solvent removed *in vacuo*. The crude material was purified by column chromatography [silicagel, $CH_2Cl_2$–acetone (30:1)] to give (**1**) (40 mg, 65%); m.p. 196—199 °C (lit.,[11] 198—202 °C); $\delta_H$($CDCl_3$, 293 K) 6.79 (1 H, s, 10-H), 6.45, 5.99, and 5.44 (6 H, AMX, $J_{AM}$ 18 Hz, $J_{AX}$ 11.7 Hz, $J_{MX}$ *ca.* 1 Hz, 2-CH=$CH_2$, 18-CH=$CH_2$), 6.00 (2 H, s, 5-H, 15-H), 3.69 (6 H, s, $CO_2Me$), 2.96 (4 H, t, *J* 7.5 Hz, 8-$CH_2$, 12-$CH_2$), 2.58 (4 H, t, *J* 7.5 Hz, $CH_2CO_2$), 2.19 and 2.12 (6 H × 2, s, 3-Me, 7-Me, 13-Me, 17-Me).

[(P,1′,R,4′R) + (M,1′S,4′S)]- *and* [(M,1′R,4′R) + (P,1′S,4′S)]-2,18-(1′-*Methoxy*-4′-*hydroxybutane*-1′,4′-*diyl*)-8,12-*bis*(2″-*methoxycarbonylethyl*)-3,7,13,17-*tetramethyl*-1,19-(21H,24H)-*bilindione* (**2a**) *and* (**2b**).—(**1**) (61 mg, 0.1 mmol) was dissolved in methanol–water–trifluroacetic acid (94:1:5) (5 ml). After 48 h at room temperature reaction was quenched with aqueous sodium hydrogen carbonate. Work-up (see above) and column chromatography on silica gel [$CH_2Cl_2$–acetone, applying a gradient from (30:1) ⟶ (30:6)] afforded starting material (**1**) (22 mg, 0.036 mmol), (**3**) (9 mg, 20%), and (**2**) (4.2 mg, 10%) in that order. For separation and characterization of individual isomers (**3a**) and (**3b**) see below. Compounds (**2**) dissolved in benzene were re-chromatographed by t.l.c. [−15 °C, toluene–ethyl acetate–propan-2-ol (75:16:9)] to give (**2a**) (3 mg, 7%, $R_F$ = 0.25) and (**2b**) (0.4 mg, 1%; $R_F$ = 0.55).

Compound (**2a**): *m/z* 658 ($M^+$); $\delta_H$($CDCl_3$, 293 K) 11.73 (1 H, br s, 22, 23-H, $D_2O$ exchangeable), 7.19 (1 H, s, 10-H), 7.03 and 6.87 (1 H × 2, br s, 21-H, 24-H, $D_2O$ exchangeable), 6.43 and 6.38 (1 H × 2, s, 5-H, 15-H), 4.44 (1 H, dd, $J_1$ 13 Hz, $J_2$ 2.5 Hz,

4′-H), 4.08 (1 H, dd, $J_1$ 13 Hz, $J_2$ 2.5 Hz, 1′-H), 3.69 (6 H, s, $CO_2Me$), 3.23 (3 H, s, 1′-OMe), 3.07 (4 H, m, 8-$CH_2$, 12-$CH_2$), 2.64 (4 H, m, $CH_2CO_2$), 2.31 and 2.29 (3 H × 2, s, 3-Me, 17-Me), 2.26 (6 H, s, 7-Me, 13-Me), *ca.* 2.2 and 1.9 (2 H and 1 H, m, 2′-H, 3′-H), 1.67 (1 H, br s, 4′-OH, $D_2O$ exchangeable), and 1.63 (1 H, m, 3′-H); $\delta_H$([$^2H_6$]benzene, 293 K) 11.60 (1 H, br s, 22, 23-H, $D_2O$ exchangeable), 7.84 and 7.41 (1 H × 2, br s, 21-H, 24-H, $D_2O$ exchangeable), 7.46 (1 H, s, 10-H), 6.06 and 5.89 (1 H × 2, s, 5-H, 15-H), 4.29 (1 H, br d, *J* 11 Hz, 4′-H), 3.81 (1 H, br d, *J* 11 Hz, 1′-H), 3.39 (6 H, s, $CO_2Me$), *ca.* 3.0 (4 H, m, 8-$CH_2$, 12-$CH_2$), 2.96 (3 H, s, 1′-OMe), 2.70 and 2.56 (2 H × 2, m, $CH_2CO_2$ × 2), 2.24 (1 H, m, 2′-H), *ca.* 2.2 (2 H, m, 2′-H, 3′-H), 2.03, 1.91, 1.89, and 1.66 (3 H × 4, s, 3-Me, 7-Me, 13-Me, 17-Me), and 1.57 (1 H, m, 3′-H).

Compound (**2b**): *m/z* 658 ($M^+$); $\delta_H$([$^2H_6$]benzene, 293 K) 12.48 (1 H, br s, 22, 23-H, $D_2O$ exchangeable), 7.98 and *ca.* 7.2 (1 H × 2, br s, 21-H, 24-H, $D_2O$ exchangeable), 7.39 (1 H, s, 10-H), 6.01 and 5.97 (1 H × 2, s, 5-H, 15-H), 4.92 (1 H, br s, 4′-H), 4.54 (1 H, br s, 1′-H), 3.39 (6 H, s, $CO_2Me$), 3.2—3.1 (4 H, m, 8-$CH_2$, 12-$CH_2$), 3.08 (3 H, s, 1′-OMe), *ca.* 2.6 (4 H, m, $CH_2CO_2$), 2.28, 2.20, 1.96, and 1.93 (3 H × 4, s, 3-Me, 7-Me, 13-Me, 17-Me), 2.2—2.0 (3 H, m, 2′-H, 3′-H), and *ca.* 1.4 (1 H, m, 3′-H).

*Deuteriation Experiments.*—If the above reaction was performed with deuteriated reagents (methan[$^2$H]ol, $D_2O$, deuteriotrifluoroacetic acid) the biliverdins (**2**) and (**3**) obtained after usual work up did not contain deuterium (n.m.r., m.s.).

[(P,1′R,4′R) + (M,1′S,4′S)]-, [(M,1′R,4′R) + (P,1′S,4′S)]-, *and* [(P,1′R,4′S) + (M,1′S,4′R)]-2,18-(1′,4′-*dimethoxybutane*-1′,4′-*diyl*)-8,12-*bis*(2″-*methoxycarbonylethyl*)-3,7,13,17-*tetramethyl*-1,19-(21H,24H)-*bilindione* (**3a**), (**3b**), *and* (**4**).—A solution of (**1**) (61 mg, 0.1 mmol) in methanolic hydrochloric acid (0.8M; 5 ml) and water (5 μl) was allowed to stand at room temperature for 48 h and then neutralized with aqueous sodium hydrogen carbonate. The residue obtained after usual work up (see above) was chromatographed on silica gel [$CH_2Cl_2$–acetone, applying a gradient from (30:1) ⟶ (30:3)] affording starting material (**1**) (5 mg, 0.008 mmol) and unseparated (**3a**), (**3b**), and (**4**) (25 mg, 40%). This mixture was dissolved in $CH_2Cl_2$ and rechromatographed using preparative t.l.c. [−15 °C, toluene–ethyl acetate–propan-2-ol (85:10:5)] to give (**3a**) (16 mg, 26%, $R_F$ = 0.12), (**3b**) (4 mg, 6%, $R_F$ = 0.40), and (**4**) (0.5 mg, 1%, $R_F$ = 0.27); in an isomer ratio (**3a**):(**3b**):(**4**) of 8:2:0.25; if the isomeric mixture was dissolved in benzene instead of $CH_2Cl_2$ prior to t.l.c. the isomer ratio was shifted in favour of (**3b**) (2:8:0.25).

Compound (**3a**): *m/z* 672 ($M^+$); u.v.–visible spectrum (ethanol) see Figure 1; $\delta_H$($CDCl_3$, 293 K) 11.62 (1 H, br s, 22, 23-H, $D_2O$ exchangeable), 7.19 (1 H, s, 10(H), 6.76 (2 H, br s, 21H, 24-H, $D_2O$ exchangeable), 6.42 (2 H, s, 5-H, 15-H), 4.10 (2 H, br d, *J* 11 Hz, 1′-H, 4′-H), 3.69 (6 H, s, $CO_2Me$), 3.23 (6 H, s, 1′-OMe, 4′-OMe), 3.08 (4 H, t, *J* 7.5 Hz, 8-$CH_2$, 12-$CH_2$), 2.64 (4 H, t, *J* 7.5 Hz, $CH_2CO_2$), 2.34 (6 H, s, 3-Me, 17-Me), 2.26 (6 H, s, 7-Me, 13-Me), *ca.* 2.2 (2 H, m, 2′-H, 3′-H), and 1.97 (2 H, m, 2′-H, 3′-H); $\delta_H$([$^2H_6$]benzene, 293 K) 11.69 (1 H, br s, 22, 23-H, $D_2O$ exchangeable), 7.50 (1 H, s, 10-H), 6.55 (2 H, br s, 21-H, 24-H, $D_2O$ exchangeable), 6.11 (2 H, s, 5-H, 15-H), 3.97 (2 H, br d, *J* 12 Hz, 1′-H. 4′-H), 3.32 (6 H, s, $CO_2Me$), 3.03 (6 H, s, 1′-OMe, 4′-OMe), 2.95 (4 H, t, *J* 7.5 Hz, 8-$CH_2$, 12-$CH_2$), 2.57 (4 H, t, *J* 7.5 Hz, $CH_2CO_2$), *ca.* 2.4 and 2.1 (2 H × 2, m, 2′-H, 3′-H), 1.98 and 1.95 (6 H × 2, s, 3-Me, 7-Me, 13-Me, 17-Me); $\delta_C$($CDCl_3$, 303 K) 173.05 ($COOCH_3$), 166.99 (1-CO, 19-CO), 113.94 (10-CH), 98.24 (5-CH, 15-CH), 75.61 (1′-CH, 4′-CH), 56.43 ($OCH_3$), 51.73 ($COOCH_3$), 35.43 ($CH_2COO$), 28.87 (2′-$CH_2$, 3′-$CH_2$), 20.17 (Ar-$CH_2$), 9.84 and 9.49 (Ar-$CH_3$). An unequivocal assignment of the respective signals for 2-, 3-, 4-, 6-, 7-, 8-, 9-, 11-, 12-, 13-, 14-, 16-, 17-, and 18-C could not be made.

Compound (**3b**): *m/z* 672 ($M^+$); u.v.–visible spectrum (ethanol) see Figure 1; $\delta_H$($CDCl_3$, 293 K) 12.57 (1 H, br s, 22, 23-H, $D_2O$ exchangeable), 7.30 (2 H, br s, 21-H, 24-H, $D_2O$ exchangeable), 7.04 (1 H, s, 10-H), 6.27 (2 H, s, 5-H, 15-H), 4.58 (2 H, br s, 1′-H, 4′-H), 3.69 (6 H, s, $CO_2Me$), 3.35 (6 H, s, 1′-OMe, 4′-OMe), 3.02 (4 H, t, *J* 7.5 Hz, 8-$CH_2$, 12-$CH_2$), 2.61 (4 H, t, *J* 7.5 Hz, $CH_2CO_2$), 2.39 (6 H, s, 3-Me, 17-Me), 2.19 (6 H, s, 7-Me, 13-Me), *ca.* 2.2 and 1.76 (2 H × 2 m, 2′-H, 3′-H); $\delta_H$([$^2H_6$]benzene, 293 K) 12.61 (1 H, br s, 22, 23-H, $D_2O$ exchangeable), 7.56 (2 H, br s, 21-H, 24-H, $D_2O$ exchangeable), 7.38 (1 H, s, 10-H), 6.03 (2 H, s, 5-H, 15-H), 4.64 (2 H, br s, 1′-H, 4′-H), 3.33 (6 H, s, $CO_2Me$), 3.05 (6 H, s, 1′-OMe, 4′-OMe), 2.98 (4 H, t, *J* 7.5 Hz, 8-$CH_2$, 12-$CH_2$), 2.57 (4 H, t, *J* 7.5 Hz, $CH_2CO_2$), *ca.* 2.4 and 2.1 (2 H × 2, m, 2′-H, 3′-H), 2.30 (6 H, s, 3-Me, 17-Me), and 1.92 (6 H, s, 7-Me, 13-Me); $\delta_C$($CDCl_3$, 303 K) 173.05 ($COOCH_3$), 168.88 (1-CO, 19-CO), 113.94 (10-CH), 97.68 (5-CH, 15-CH), 79.35 (1′-CH, 4′-CH), 56.93 ($OCH_3$), 51.73 ($COOCH_3$), 35.43 ($CH_2COO$), 27.36 (2′-$CH_2$, 3′-$CH_2$), 20.17 (Ar-$CH_2$), 9.84 and 9.49 (Ar-$CH_3$); an unequivocal assignment of the respective signals for 2-, 3-, 4-, 6-, 7-, 8-, 9-, 11-, 12-, 13-, 14-, 16-, 17-, and 18-C could not be made.

Compound (**4**): *m/z* 672 ($M^+$); $\lambda_{max.}$(benzene, 20 °C) 657 (ε 16 000), 385 (43 300), 310 (28 300); $\delta_H$($CDCl_3$, 293 K) 12.03 (1 H, br s, 22, 23-H, $D_2O$ exchangeable), 7.12 (1 H, s, 10-H), 7.12 and 6.97 (1 H × 2, br s, 21-H, 24-H, $D_2O$ exchangeable), 6.38 and 6.32 (1 H × 2 s, 5-H, 15-H), 4.50 (1 H, br s, 4′-H), 4.05 (1 H, br d, *J ca.* 11 Hz, 1′-H), 3.70 (6 H, s, $CO_2Me$), 3.39 and 3.27 (3 H × 2, s, 1′-OMe, 4′-OMe), 3.06 (4 H, m, 8-$CH_2$, 12-$CH_2$), 2.64 (4 H, m, $CH_2CO_2$), *ca.* 2.3 and 2.0 (4 H, m, 2′-H, 3′-H), 2.29, 2.22, 2.22, and 2.19 (3 H × 4, s, 3-Me, 7-Me, 13-Me, 17-Me).

*Derivation of* (**3a**) *and* (**3b**) *from* (**2a**) *and* (**2b**).—A solution of (**2**) (3 mg, 4.5 μmol) in methanolic hydrochloric acid (0.8M; 0.5 ml) was allowed to stand 24 h at room temperature. Usual work up and t.l.c. (for eluants see above) afforded (**3a**) and (**3b**) (2.5 mg, 82%); for u.v.–visible, n.m.r., and mass spectra see above.

*Influence of Initial Concentration of* (**1**) *on the Rate of Formation of* (**3**) *and Cross Experiments with Biliverdin*-IXα *Dimethyl Ester* (BVE-IXα).—Runs 1—4 (Figure 3) were performed with methanolic hydrochloric acid (0.8M; 0.1% water) (0.5 ml) and initially contained 3 mg (**1**) (run 1), 0.61 mg (**1**) (run 2), 0.61 mg (**1**) + 2.4 mg (BVE-IXα) (run 3), and 3 mg (**1**) + 12.2 mg (BVE-IXα) (run 4). After 16, 20, 24, and 38 h samples were taken from all runs. After the usual work up the solutions of runs 1—4 were appropriately diluted with $CH_2Cl_2$ taking into account the initial concentration of (**1**) and subjected to t.l.c. [−5 °C, toluene–ethyl acetate–propan-2-ol (85:10:5)]. The densitometer readings of the zone corresponding to (**3a**) (λ 630 nm) were directly proportional to the yield and were corrected for additional (**3b**) formed.

*Equilibration Experiments.*—Isomers [(**3a**) + (**3b**)] and (**4**) (each 0.5 mg) were separately dissolved in [$^2H_4$]methanol–water–sulphuric acid (95:0.2:4.8) (0.5 ml) and allowed to stand for 24 h at room temperature. After the usual work up materials were subjected to t.l.c. No (**4**) was detected in the run starting from [(**3a**) + (**3b**)] and *vice versa* (recovery 85%). The $^1H$ n.m.r.s revealed complete displacement of ether- and ester-methyl groups against trideuteriomethyl groups for both runs. Performing these reactions with trifluoroacetic acid instead of sulphuric acid the same results were obtained.

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