Novel Reaction Modes of Biliverdin-IXα: Synthesis and Structure of Bilatriene Dimers

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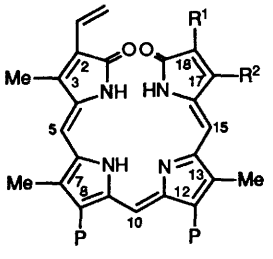
Novel reactions of biliverdin-IXα dimethyl ester **2** affording compounds carrying two bilatriene entities are reported. Thus compound **2** undergoes various acid-promoted dimerisations by regio- and site-selective reaction of the respective vinyl groups adjacent to the pyrrolinone carbonyls to give the cyclobutane **4**, the diastereoisomeric cyclohexenes **5a** and **5b**, the butene **6** and its corresponding methanol adducts **7a** and **7b**. The distribution of products can be controlled by varying the concentration of acid and temperature. The elucidation of structure, stereochemistry and conformation of the compounds synthesised is accomplished mainly using spectroscopic means. The 1,2-*trans* relation of the two biliverdin moieties in the cyclobutane **4** is established by an asymmetric synthesis. The mechanism of reactions and the differences in the reactivity of the two vinyl groups in compound **2** and other biliverdins are discussed.

In an earlier work we reported on the synthesis[1] and resolution[2] of a C_4-bridged tetrapyrrolic macrocycle from biliverdin-IIIα dimethyl ester **1**. The formation of the four-link chain between the pyrrolinone rings is initiated by reaction of the two equivalent vinyl groups at positions 2 and 18. Biliverdin-IXα dimethyl ester **2**, showing the natural substitution pattern, differs from compound **1** by permutation of the methyl and vinyl substituents of one pyrrolinone ring. An intramolecular reaction involving the two differently positioned vinyl groups is not observed. However, as one vinyl group in compound **2** (2-Vn)† is still in the same position as in compound **1** an intermolecular reaction between two molecules of compound **2** might be possible.



1 R^1 = $CH{=}CH_2$, R^2 = Me
2 R^1 = Me, R^2 = $CH{=}CH_2$
P = $CH_2CH_2CO_2Me$

Owing to its importance in nature as an intermediate in haeme catabolism[4,5] and its current use as a model for the cofactor of biliproteins[5,6] the reaction modes of biliverdin-IXα and its derivatives are of general interest. This prompted us to undertake a systematic investigation into the reactivity of vinyl groups of bilatrienes. In this report we describe some unexpected and hitherto unknown reactions of biliverdin-IXα, which furnish novel bichromophoric bile pigments, and then delineate their mode of formation.

† Note that numbering of compound **2**, although still unequivocal, does not completely conform with that recommended (ref. 3), starting with the pyrrolinone ring which carries the *exo*-vinyl group. This slight modification bears the advantage that numbers of C-positions are retained in the reaction products **4**–**7**, thus avoiding confusion in reading the paper.

Results and Discussion

Syntheses.—All dimerisations of compound **2** described are acid catalysed and are initiated by regioselective intermolecular bond formation between the two 2-vinyl groups. Under all conditions employed the 17-vinyl group resists reaction. The corresponding products could not be detected, even trace amounts. The reaction mode essentially depends upon the amount of acid in excess over compound **2**.

If this excess is low *two* new C–C bonds are formed. Accordingly, at ambient temperature formal head-to-head (2 + 2) cycloaddition predominates, stereospecifically furnishing the cyclobutane **4**, a C_2-linked bilatriene dimer. This reaction proceeds in most solvents [dimethyl sulfoxide (DMSO), tetrahydrofuran (THF), chloroform, alcohols, ethyl lactate] containing 1–10 mol equivalents of sulfuric acid or any sulfonic acid. However, the rate of reaction is considerably lower than that observed for the intramolecular reaction of compound **1**.[1] Therefore, in order to obtain synthetically valuable rates at all, the initial concentration of compound **2** must be large (>0.1 mol dm^{-3}) so that the solubility comprises the limiting factor of the medium considered. Best results are obtained by using methanol–(1*S*)-(+)-camphor-10-sulfonic acid (CSA),‡ allowing the initial concentration in substrate **2** to be as high as 0.15 mol dm^{-3}. Even under these more optimised conditions and the long reaction time (40 days), 90% of the starting material were recovered (yield 26%). Doubling of the reaction time increased only the amount of by-products and did not improve the yield. The isomeric cyclohexenes **5a** and **5b** are obtained as minor components (9%). The product proportions§ **4**:**5a**:**5b** are 14:2:3, the overall yield being 35%. As the temperature is raised to 65 °C, rate of conversion increases but simultaneously the formation of compounds **5** is favoured at the expense of the cyclobutane **4**.¶ Thus, after 24 h, the proportions are 5:5:7 (40% recovery of **2**, total yield 48%). However, variation of tempera-

‡ Note that CSA is used only to increase the solubility of compound **2**. Its optical activity is irrelevant for the reaction *per se*. The products formed (**4**, **5a** and **5b**) are throughout racemic. For an asymmetric synthesis see below.

§ Here, and in the following text, the product proportions given refer to unseparated mixtures and have been determined by ^1H NMR spectroscopy; they are in good agreement with those obtained after chromatography.

¶ At elevated temperatures sulfuric acid gave fewer decomposition products than did CSA.

Table 1 ^{1}H NMR data[a–c] of the biliverdin (BV) and hydrobiliverdin (HBV) moieties of compounds **2** and **4**–**7** and their assignment[d]

	2[e] BV	**4** BV	**5a** HBV	**5a** BV	**5b** HBV	**5b** BV
10-H	6.77 (1 H, s)	6.66 (2 H, s)	6.69 (1 H, s)	6.70 (1 H, s)	6.57 (1 H, s)	6.66 (1 H, s)
15-H	6.05 (1 H, s)	5.98 (2 H, s)	6.18 (1 H, s)	5.96 (1 H, s)	6.10 (1 H, s)	5.93 (1 H, s)
5-H	6.00 (1 H, s)	5.75 (2 H, s)	5.48 (1 H, s)	5.84 (1 H, s)	5.14 (1 H, br s)	5.76 (1 H, br s)
17-Vn-H_X	6.63 (1 H, m)	6.62 (2 H, m)	6.67 (1 H, m)	6.59 (1 H, m)	6.64 (1 H, m)	6.56 (1 H, m)
17-Vn-H_{AB}	5.65 (2 H, m)	5.64 (4 H, m)	~5.6 (4 H, m)		~5.6 (4 H, m)	
CO_2Me	3.66 (6 H, s)	3.65 (6 H, s), 3.64 (6 H, s)	3.67–3.64 (3 H × 4, s)		3.64–3.60 (3 H × 4, s)	
8-, 12-CH_2	2.92 (4 H, m)	2.87 (8 H, m)	~2.9 (8 H, m)		~2.9 (8 H, m)	
CH_2CO_2	2.54 (4 H, m)	2.52 (8 H, m)	~2.5 (8 H, m)		~2.5 (8 H, m)	
18-Me	1.85 (3 H, s)	1.94 (6 H, s)	1.94 and 1.87 (3 H × 2, s)		1.89 and 1.78 (3 H × 2, s)	
13-Me	2.09 (3 H, s)	2.04 (6 H, s)	2.11 (3 H, s)	2.03 (3 H, s)	2.06 (3 H, s)	2.02 (3 H, s)
7-Me	2.06 (3 H, s)	2.00 (6 H, s)	1.91 (3 H, s)	2.05 (3 H, s)	1.95 (3 H, s)	1.98 (3 H, s)
3-Me	2.17 (3 H, s)	1.97 (6 H, s)	1.41 (3 H, s)	2.09 (3 H, s)	1.63 (3 H, s)	~2.0 (3 H, br s)

	6 BV	**7a** BV	**7b** BV
10-H	6.73 and 6.66 (1 H × 2, s)	6.65 and 6.62 (1 H × 2, s)	6.66 (2 H, s)
15-H, 5-H	6.02, 5.94, 5.91 and 5.81 (1 H × 4, s)	5.92, 5.91, 5.83 and 5.72 (1 H × 4, s)	6.00, 5.97, 5.86 and 5.81 (1 H × 4, s)
17-Vn-H_X	6.56 and 6.54 (1 H × 2, m)	6.53 (2 H, m)	6.61 and 6.57 (1 H × 2, m)
17-Vn-H_{AB}	~5.6 (4 H, m)	~5.6 (4 H, m)	~5.6 (4 H,)
CO_2Me	~3.6 (12 H, s)	~3.6 (12 H, s)	3.68, 3.67, 3.66 and 3.65 (3 H × 4, s)
8-, 12-CH_2	~2.9 (8H, m)	~2.8 (8 H, m)	~2.9 (8 H, m)
CH_2CO_2	~2.5 (8 H, m)	~2.5 (8 H, m)	~2.5 (8 H, m)
18-Me, 13-Me, 7-Me, 3-Me	2.11, 2.06, 2.02, 2.01, 1.99, 1.99, 1.85 and 1.81 (3 H × 8, s)	2.08, 1.99, 1.99, 1.99, 1.98, 1.97, 1.84 and 1.84 (3 H × 8, s)	2.14, 2.08, 2.06, 2.05, 2.02, 1.99, 1.93 and 1.91 (3 H × 8, s)

[a] Chemical shifts (δ) for ~10^{-2} mol dm^{-3} solutions. [b] 250 MHz (**2**, **4** and **5**) and 400 MHz (**6** and **7**), respectively, 297 K, in $CDCl_3$. [c] For the spectrum of compound **4** in [$^2H_5$]pyridine see Experimental section. [d] Assignments by COSY, ^{1}H-detected ^{1}H–^{13}C shift correlation and NOE difference spectra. [e] 2-Vn-H_X, δ 6.50 (1 H, m), 2-Vn-H_M 6.13 (1 H, m), 2-Vn-H_A 5.44 (1 H, m).

ture has no significant influence on the isomer ratio **5a**:**5b**, it being still ~2:3. If the initial concentration in substrate **2** is lowered, the rate of formation of dimers **4** and **5** decreases and the methoxybiliverdin **3** appears. At concentrations less than 0.01 mol dm^{-3} dimerisation can no longer be observed. Compounds **5** can also be obtained by treatment of the cyclobutane **4** with acidic methanol at 65 °C. Remarkably, the isomer ratio **5a**:**5b** (~ 1:10) significantly differs from that found *via* compound **2** when using the same concentration in acid and the same temperature. This implies that at least two different mechanisms account for the formation of products **5** (see below).

If the reaction medium becomes increasingly acidic, *eg.* by the use of mixtures of methanol–sulfuric acid from 10:1 to 1:10, the amount of products **4** and **5** drastically decreases and other dimers, compounds **6** and **7**, arising from a distinctly different reaction mode, appear. Owing to considerable decomposition and the competitive formation of the methoxybiliverdin **3** yields are low under these conditions. However, synthesis of the C_3-linked bilatriene dimers **6** and **7** proceeds smoothly if compound **2** is dissolved in conc. sulfuric acid followed by quenching with methanol after 5 min. If the quenching temperature is low (−20 °C) the diastereoisomeric methanol adducts **7a** and **7b** are the main products formed (ratio ~ 1:1, yield 70%), while at 65 °C the butene **6** predominates (yield 41%). Accordingly, this reaction mode affords dimers with only *one* new C–C bond between monomeric entities. Products corresponding to anti-Markovnikov orientation are not observed. If the reaction time of compound **2** in sulfuric acid exceeds 5 min the formation of oligomers drastically reduces the yield.

Structure and Stereochemistry.—Field desorption (FDMS) and fast-atom bombardment mass spectra (FABMS) clearly show that compounds **4**–**7** are dimers of compound **2**; in compounds **7** 1 mol of methanol is found additionally. The two tetrapyrrole moieties of compounds **4**, **6**, **7a** and **7b** are almost indistinguishable from that of the precursor **2** with respect to ^{1}H and ^{13}C chemical shifts (Tables 1 and 2). Only the corresponding 2-vinyl groups (2-CH=CH_2, δ_C 119.9) formerly present in the two monomeric consitituents, which characteristically resonate at higher field than the 17-vinyl groups (17-CH=CH_2, δ_C 122.0), have disappeared (Table 2). Dimers **5**, however, are each composed of two distinctly different tetrapyrrole moieties as can be seen from their ^{1}H and ^{13}C NMR spectra. One of them is still a biliverdin moiety as found for compounds **4**, **6** and **7**. Concerning the other chromophore of compounds **5a** and **5b**, C(H)-5 (δ_C 91.75 and 91.35, respectively) and C(H)-10 (δ_C 111.68 and 111.92, respectively) resonate at exceptional high field while the chemical shift for C(H)-15 (δ_C 97.68 and 98.06, respectively) is similar to that observed for the biliverdin chromophore. These shift values are typical for bilatrienes in which conjugation within one pyrrolinone ring is disturbed.[7] Again, as follows from the CH=CH_2 resonance absorptions, the 17-vinyl groups are still present. The preservation of the 17-vinyl groups in the cyclobutane **4** (15′-H ⟶ 17′-Vn-H_X) and compounds **5a** and **5b** (15-H ⟶ 17-Vn-H_X, 15″-H ⟶ 17″-Vn-H_X) is further corroborated by NOE experiments.*

Having assigned the structure of the chromophores present in the dimers the following discussion will be devoted mostly to the links connecting them, including the symmetry and stereo-

* The double prime positions refer to the respective biliverdin residue (BV) of compounds **5** (non systematic numbering).

Table 2 ^{13}C NMR data[a,b] of the biliverdin (BV) and hydrobiliverdin (HBV) moieties of compounds **2** and **4**–**7** and their assignment[c,d]

	2[e] BV	**4** BV	**5a** HBV	**5a** BV	**5b** HBV	**5b** BV
CO_2Me	173.06–	173.05–	173.29– and	173.19–	173.51– and	173.14–
CO-1	172.19–	172.15–	167.68–	172.74–	168.38–	~172.6–
CO-19	171.31–	172.15–	172.25– and	172.74–	~172.6–	
C-2			124.08–		123.88–	
C-3			45.30–		44.28–	
17-CH=CH_2	126.60+	126.43+	126.59+	126.26+	126.49+	126.12+
17-CH=CH_2	122.03–	122.61–	122.54–		122.38– and	122.26–
CH-10	114.43+	114.80+	111.68+	114.95+	111.92+	114.85+
CH-15	97.63+	98.03+	97.68+	97.52+	98.06+	
CH-5	97.63+	96.48+	91.75+	96.66+	91.35+	96.50+
CO_2Me	51.65+	51.70+	~51.7+		51.42+	
CH_2CO_2	35.21–	35.17–	35.14– and	35.04–	35.00–	
8-, 12-CH_2	19.84–	19.79–	19.67–		19.56–	
3-Me	9.49+, 9.32+	9.51+, 9.40+	24.53+	10.68+	32.30+	10.5+
7-, 13-, 18-Me			9.40+, 9.26+ and 9.07+		~9.1+	

	6 BV	**7a** BV	**7b** BV
CO_2Me	173.20–	173.17– and 173.15–	173.25– and 173.22–
CO-1, CO-19	172.64– and 172.11–	172.61–, 172.53–, 171.97– and 171.50–	172.48–, 172.37–, 171.92– and 171.46–
C-2			
C-3			
17-CH=CH_2	126.13+	126.38+ and 126.33+	126.15+ and 126.11+
17-CH=CH_2	122.48–	122.52– and 122.48–	122.42–
CH-10	114.90+ and 114.60+	114.88+ and 114.62+	114.80+ and 114.64+
CH-15, CH-5	97.98+, 97.98+, 97.32+ and 96.75+	97.69+, 97.62+, 97.06+ and 95.86+	98.01+, 97.88+, 97.25+ and 96.18+
CO_2Me	51.48+	51.64+	51.48+
CH_2COO	34.95–	35.12–	34.98– and 34.95–
8-, 12-CH_2	19.64– and 19.60–	19.76–	19.61–
3-Me	9.78+, 9.35+ and 9.11+	9.39+ and 9.33+	9.24+, 9.16+ and 9.10+
7-, 13-, 18-Me			

[a] Chemical shifts (δ) for ~10^{-2} mol dm^{-3} solutions; signs refer to the signs of signals in *J*-modulated ^{13}C spectra. [b] 62.9 MHz; 313 K, in $CDCl_3$–[$^2H_4$]methanol (1:9). [c] Assignments by comparison with estimated shift values using the 'CSEARCH'-database[17] and ^1H-detected ^1H–^{13}C shift-correlation experiments. [d] Carbons not listed have not been assigned. [e] In $CDCl_3$, 303 K. [f] 2-CH=CH_2, δ_C 125.73+; 2-CH=CH_2, δ_C 119.88–.

chemistry of the whole molecules. In the cyclobutane **4** a C_2 axis or a plane of symmetry must be present as follows from the chemical equivalence of the two biliverdin moieties found in the ^1H and ^{13}C NMR spectra. In the aliphatic absorption region only three additional proton signals appear (proportions 1:1:1; see Table 3 and Experimental section). One of them correlates with an aliphatic CH, the other two with an aliphatic CH_2 ^{13}C resonance absorption. These findings suggest that the spacer between the two biliverdin moieties is a cyclobutane ring. Five isomeric disubstituted cyclobutanes can be envisaged for the structure of compound **4**. The presence of three chemically different sorts of protons found for the cyclobutane link safely excludes the 1,1- and the *trans*-1,3-isomer. Addition of two equivalents of (*R*)-(−)-mandelic acid–forming diastereoisomeric salt-like associates with bilatrienes[2]–to a solution of compound **4** in benzene–chloroform results in a splitting of most ^1H NMR signals in a 1:1 ratio. This phenomenon can be expected to occur only for each of the two 1,2-isomers bearing two chiral centres. Thereby the *cis*-isomer [configuration (1*R*,2*S*)] represents a *meso* form while the *trans*-isomer is chiral, consisting of two equipopulated enantiomers of configurations (1*R*,2*R*) and (1*S*,2*S*). Evidence for the 1,2-*trans*-stereochemistry was obtained by partial resolution of compound **4** *via* an asymmetric synthesis from compound **2**. Best results were obtained with (*S*)-(−)-ethyl lactate containing 10 mol equivalents of sulfuric acid. Under these conditions dimerisation was accompanied by partial replacement of the methyl ester groups, and mixed ethyl lactate and ethyl esters were formed during the reaction time (20 days). The mixture obtained was therefore reconverted into the methyl esters by treatment with methanol–sulfuric acid (5% v/v) for 2 h. This procedure gave partially resolved optically active (+)-**4**, $[\alpha]_{436}^{20}$ +300 10^{-1} deg cm^2 g^{-1}; $\Delta\varepsilon$ (645 nm) −2.3. The enantiomeric excess (ee) was evaluated by ^1H NMR spectroscopy to be 14% (see Experimental section). No effort has been made to optimize the enantioselectivity. Compounds **5** were not formed under these conditions.

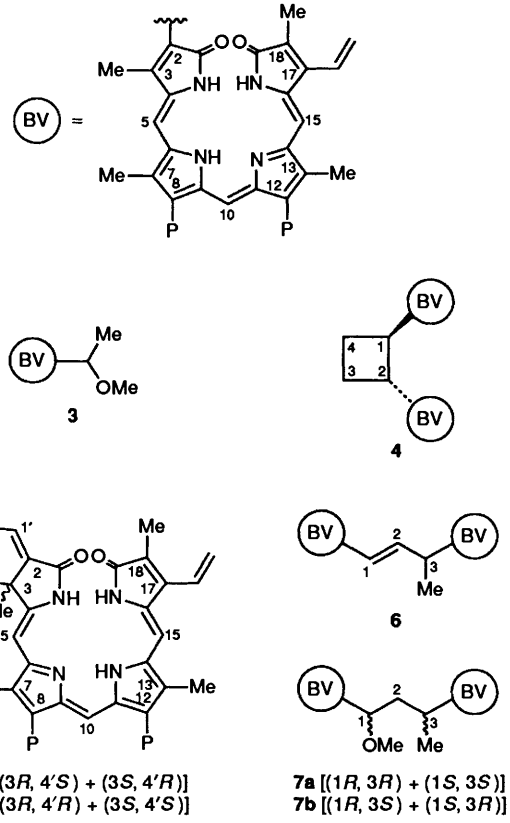
The quotient of dipole strengths (*f* 2.14) of the two main UV–VIS absorption bands of compound **4** is similar to that of compound **3** (*f* 2.28) which also lacks the 2-vinyl group. In addition the spectrum is closely similar to those of compounds **7a** and **7b** (Fig. 1) and can be composed from that reported[8] for 2-devinyl-2-ethylbiliverdin-IXα dimethyl ester. On the other hand *f* comprises a sensitive monitor for conformational changes in open-chain tetrapyrroles.[6] Accordingly, since compound **3**, like most unrestricted bilatrienes, preferentially adopts a *Z,Z,Z,syn,syn,syn* conformation,[5] a helical coiled arrangement is most likely for the chromophores of compounds **4** and **7**, too. This is fully corroborated by the NOE enhancements 3′-Me ⟷ 5′-H; 5′-H ⟷ 7′-Me; 8′-, 12′-CH_2 ⟷ 10′-H; 13′-Me ⟷ 15′-H; and 15′-H ⟶ 17′-Vn-H_X observed for compound **4**. Owing to the two-fold axis the relative orientation of chromophores in space could not be determined by NMR analysis.

The moieties linking the structurally different bilatrienes in compounds **5a** and **5b** are more difficult to analyse. This is mainly due to occasional overlapping of NMR resonance

Table 3 ^{1}H and ^{13}C NMR data[a–c] for the link between the biliverdin moieties of compounds **2** and **4–7**

Compound	
4	δ_H 3.90 (2 H, m, 1- and 2-H), 2.46 and 2.05 (2 H × 2, m, 3- and 4-H) δ_C 35.86+ (CH-1 and CH-2), 23.49− (CH_2-3 and CH_2-4)
5a[d]	δ_H 6.49 (1 H, t, $J_{1',2'}$ 3.5, 1'-H), 3.18 (1 H, dd, $J_{4',3'a}$ 12.5, $J_{4',3'b}$ 3.6, 4'-H), 2.85 (1 H, m, 3'-H_a), 2.40 (2 H, m, 2'-H_2), 1.70 (1 H, m, 3'-H_b) δ_C 131.70+ (CH-1'), 39.19+ (CH-4'), 24.73− (CH_2-2'), 21.66− (CH_2-3')
5b[d]	δ_H 6.71 (1 H, t, J ~3, 1'-H), 3.44 (1 H, br m, 4'-H), 2.30 (2 H, m, 2'-H_2), 2.25 (1 H, m, 3'-H_a), ~1.8 (1 H, m, 3'-H_b) δ_C 132.80+ (CH-1'), 36.21+ (CH-4'), 22.72− (CH_2-2' and CH_2-3')
6	δ_H 6.83 (1 H, dd, $J_{2,1}$ 16, $J_{2,3}$ 7, 2-H), 6.17 (1 H, d, $J_{1,2}$ 16, 1-H), 3.49 (1 H, quintet, J 7, 3-H), 1.30 (3 H, d, J 7, 3-Me) δ_C 138.57+ (CH-2), 118.50+ (CH-1), 33.76+ (CH-3), 17.79+ (3-Me)
7a	δ_H 4.05 (1 H, dd, $J_{1.2a}$ 8, $J_{1.2b}$ 6.5, 1-H), 3.15 (3 H, s, 1-OMe), 2.74 (1 H, sextet, J 7–8, 3-H), 2.17 (1 H, m, 2-H_a), ~2.0 (1 H, m, 2-H_b), 1.13 (3 H, d, J 7, 3-Me) δ_C 73.77+ (CH-1), 56.54+ (1-OMe), 38.18− (CH_2-2), 27.41+ (CH-3), 18.35+(3-Me)
7b	δ_H 3.96 (1 H, dd, $J_{1.2a}$ 9, $J_{1.2b}$ 6, 1-H), 3.20 (3 H, s, 1-OMe), 2.95 (1 H, m, 3-H), 2.2–2.0 (1 H × 2, m, 2-H_a and 2-H_b), 1.24 (3 H, d, J 7, 3-Me) δ_C 74.03+ (CH-1), 56.37+ (1-OMe), 38.70− (CH_2-2), 27.27+ (CH-3), 18.76+(3-Me).

[a,b] See footnotes *a,b* to Tables 1 and 2. [c] Assignments see footnote *d* to Table 1 and footnote *c* to Table 2. [d] See also Fig. 2.



Only one enantiomer of compound **4** is shown. Stereochemical assignment for stereoisomers **7a** and **7b** is arbitrary. For systematic numbering of compounds **5** see Experimental section.

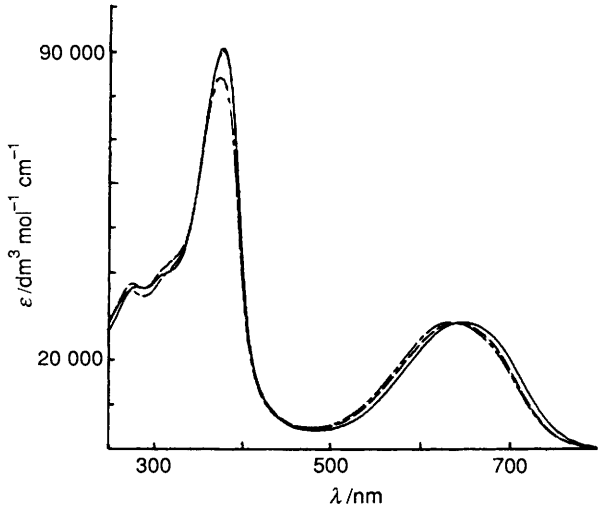


Fig. 1 UV–VIS spectra of compounds **4** (——), **7a** (– – –) and **7b** (–·–·–)

absorptions. Compounds **5** are very similar with respect to all spectroscopic properties observed (Tables 1–3), including their UV–VIS spectra (see Experimental section), which suggests only slight structural differences between them. The link connecting the two chromophores shows five aliphatic and one olefinic proton. One methyl group of the bilatriene chromophore which has suffered structural changes is found in an aliphatic position (C-3). The intimate details of structures were obtained from a combined evaluation of 2D-NMR spectra (COSY, ^{1}H-detected ^{1}H–^{13}C shift correlation) and NOE experiments. Results on the most important structural fragments are as depicted in Fig. 2. Compounds **5a** [(3*R*,4'*S*) + (3*S*,4'*R*); *cis*] and **5b** [(3*R*,4'*R*) + (3*S*,4'*S*); *trans*] represent diastereoisomeric pairs of enantiomers differing in the relative orientation of the methyl group at C-3 and the biliverdin moiety at C-4'. The stereochemistry proposed (**5a**: *cis* and **5b**: *trans*) follows from the NOE enhancements 4'-H ⟷ 3-Me observed for isomer **5b**. There is an additional difference between the two isomers: while compound **5a** exhibits well resolved ^{1}H NMR spectra even down to 233 K many signals of isomer **5b**, especially the methine protons 5-H and 5″-H, the aliphatic 4'-H, and 3″-Me, are exceptionally broad at room temperature. This phenomenon essentially persists if the temperature is raised to 373 K. On the other hand at low temperature (253 K) a splitting of many signals in an approximate 1:1 ratio occurs. Largest differences in chemical shifts are observed for the methine protons 5-H and 5″-H. The signals due to 4'-H and 3″-Me cannot be seen due to interference by other absorptions. Assuming that only two species are involved in this dynamic process the free enthalpy of activation for interconversion is estimated to be ~60 kJ mol^{-1}. Three possibilities for the origin of this apparent inhomogeneity can be envisaged: (i) twist-boat conformers of the cyclohexene moiety, (ii) hindered interconversion of one of the bilatriene helices and (iii) rotamers due to steric hindrance of internal rotation around the C-4'–C-2″ single bond. At the present time none of these alternatives can safely be excluded.

UV–VIS spectra of isomers **5** are less informative since both comprise an envelope of two subspectra arising from differently absorbing chromophores. However, when we made the plausible assumption that the biliverdin moieties in **5a** and **5b** are helically arranged (see above), their spectra could then be further analysed by subtracting the contribution from one

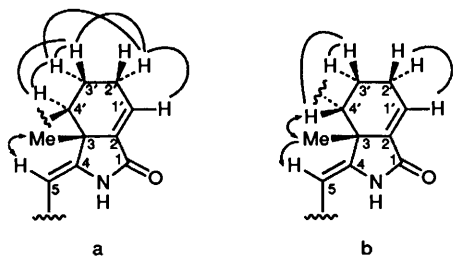


Fig. 2 Structure and stereochemistry of isomers **5a** (a) and **5b** (b) in the vicinity of the cyclohexene fragment. Only one enantiomer is shown. Assignments by COSY (lines), NOE enhancements (arrows) and ^{1}H-detected ^{1}H–^{13}C chemical-shift correlation (see also Tables 1–3). Non-systematic numbering.

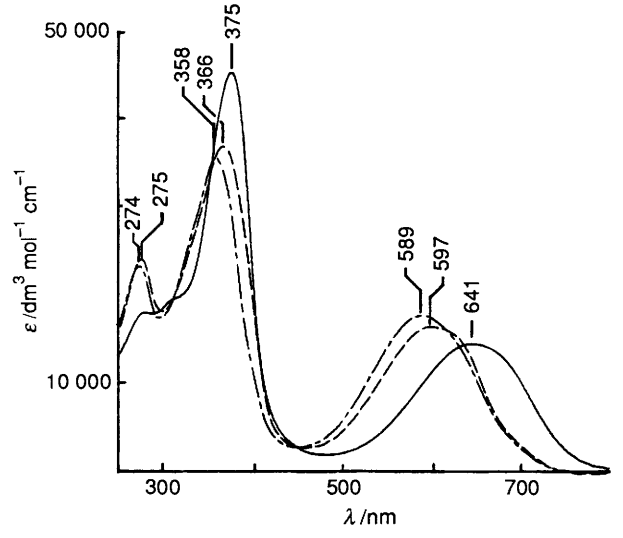


Fig. 3 UV–VIS difference spectra obtained from isomers **5a** (– – –) and **5b** (– · – · –), respectively, by subtraction of the contribution from one biliverdin chromophore of compound **4** (——)

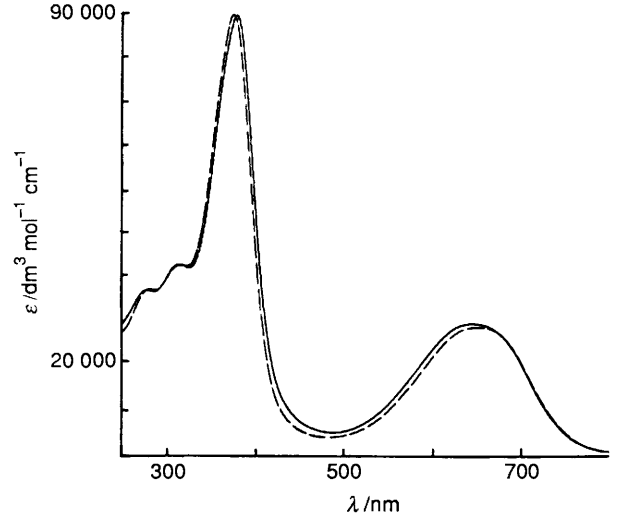


Fig. 4 UV–VIS spectrum of compound **6** (——) and its composition from the spectra of compound **2**[6] and one biliverdin chromophore of compound **4** (– – –)

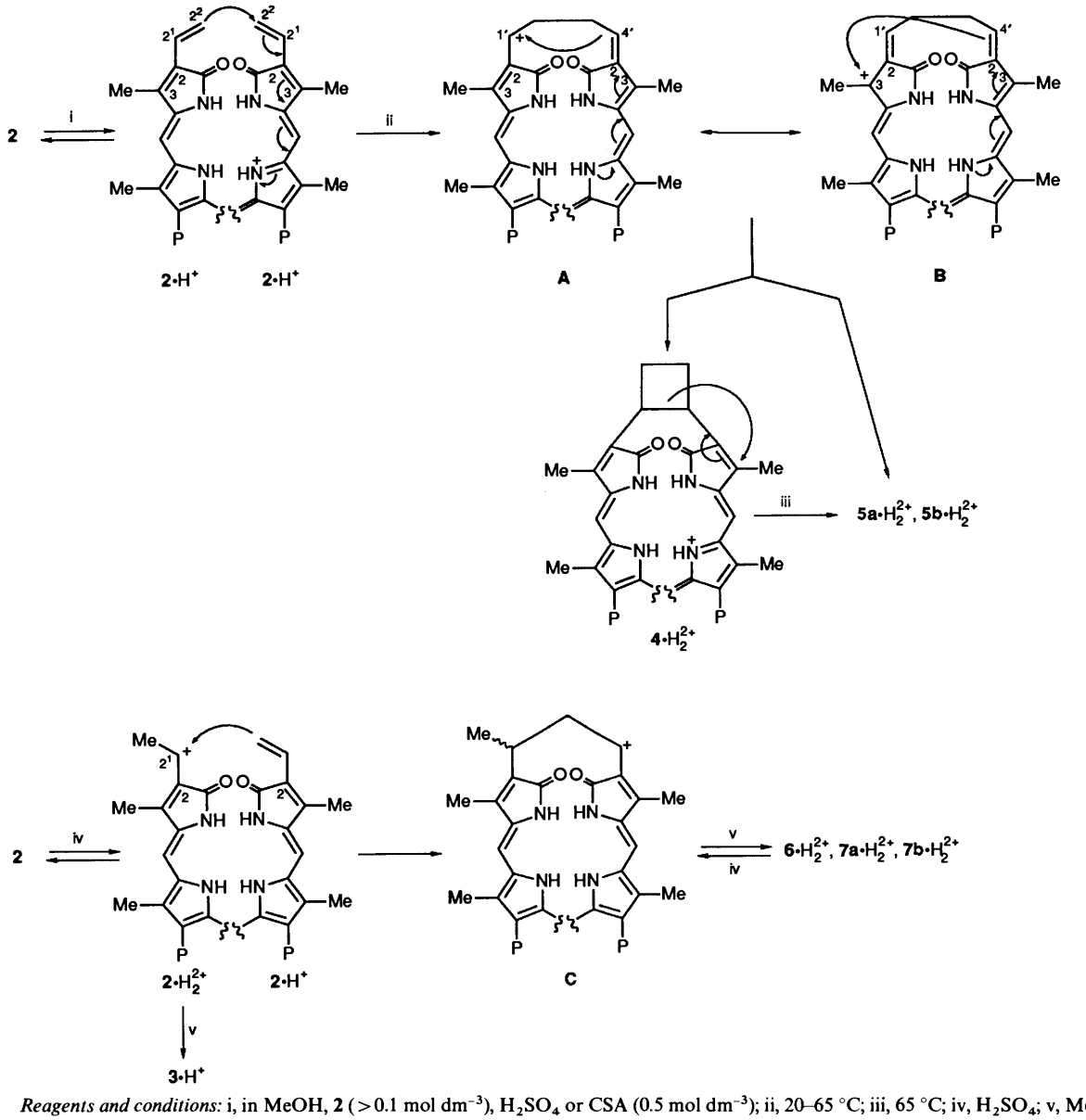
biliverdin chromophore as present in compound **4**. This gives difference spectra characteristic for helical hydrobiliverdins[4,5,9] with interrupted conjugation along the C-4–C-3–C-2 carbons (Fig. 3). Further evidence in support of the *Z,Z,Z,syn,syn,syn* conformations of the bilatriene moieties was obtained from NOE difference spectra performed on isomer **5a** (3-Me ⟷ 5-H; 5-H ⟷ 7-Me; 13-Me ⟷ 15-H; 15-H ⟶ 17-Vn-H_X; 3″-Me ⟷ 5″-H; 5″-H ⟷ 7″-Me; 13″-Me ⟷ 15″-H; 15″-H ⟶ 17″-Vn-H_X) and isomer **5b** (3-Me ⟶ 5-H; 13-Me ⟷ 15-H; 15-H ⟶ 17-Vn-H_X; 5″-H ⟷ 7″-Me; 13″-Me ⟷ 15″-H; 15″-H ⟶ 17″-Vn-H_X). Owing to severe overlapping in the absorption region of the methylene groups of the propionic side-chains the spatial relationship between rings B and C, and B″ and C″, respectively, could not be determined. Additionally, in isomer **5b** some NOE enhancements could not be detected due to exceptional broadness of signals (3″-Me, 5-H; see above).

The interchromophoric NOEs obtained for isomers **5a** (3″-Me ⟶ 5-H) and **5b** (3-Me ⟶ 5″-H) show some additional interesting features concerning the relative orientation of the two bilatriene entities. They are differently arranged, being situated in approximately parallel (**5a**) and orthogonal planes (**5b**), respectively. However, owing to the conformational heterogeneity suggested for isomer **5b** (see above) the spatial relation derived might be valid only for one species.

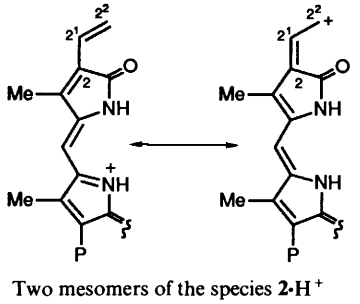
The two identical biliverdin moieties of compounds **6** and **7** are unsymmetrically placed within their respective molecules. The structure of the corresponding C_3-links simply follows from the data in Table 3. The assignment of the configuration of the $\Delta^{1,2}$ double bond in compound **6** (*E*) is based on the coupling constant (J_{AB} 16 Hz) between the two olefinic protons. Structures **7a** and **7b** represent the corresponding diastereoisomeric methanol adducts of configurations (1*R*,3*S*) + (1*S*,3*R*) and (1*R*,3*R*) + (1*S*,3*S*) or *vice versa*. No effort has been made to attempt their stereochemical differentiation. UV–VIS spectra and *f*-values of compounds **6** (*f* 2.40), **7a** (*f* 2.16) and **7b** (*f* 2.01) are indicative of a helical arrangement of chromophores. The spectrum of compound **6** can be composed from the spectra of the individual chromophores present in compounds **2** and **4** (Fig. 4). For UV–VIS spectra of stereoisomers **7** and their comparison with that of the cyclobutane **4** see Fig. 1. NOE experiments were prohibited by the similarity of resonance absorptions of the two almost identical chromophores.

Mechanism.—Under all conditions employed compound **2** is fully protonated by the amount of the strong acid present, as follows from p*K*-values reported for bilatrienes.[10] No conversions are observed in neutral solution. Thus **2**·H^+ rather than **2** itself must be considered as the reactive species. The pronounced differences in reactivity of the 2-(18-) and 17-(3-) vinyl groups can be ascribed to differences in conjugative interactions with the bilatriene double bonds. Accordingly, any movement of π-electrons of the 2-vinyl group can proceed with participation of the whole π-electron system of the bilatriene backbone stabilising a positive charge at both vinylic positions, C-2^1 and C-2^2. This is important for the stabilisation of (i) intermediates **A**, **B** and **C**, (ii) the doubly protonated intermediate species **2**·H_2^{2+} and (iii) the vinylogous cation **2**·H^+ (Schemes 1 and 2). In general similar properties can be expected for vinyl at C-8 (C-12), while if located at C-7 (C-13) a positive charge can be stabilised at only one vinylic position (C-7^1, C-13^1). For vinyl substituents at C-17 (C-3), however, stabilisation of a positive charge at either vinylic position by conjugation with the bilatriene double bonds would produce energetically unfavourable mesomeric structures, in which only the pyrrolinone moiety becomes involved, otherwise separation of charge would occur. In other words the cross-conjugation point at C-17 (C-3) prevents effective conjugation of the vinyl group with the tripyrrolic entity.* Consequently any reaction of the 17-(3-)

* The concept of cross conjugation has been applied by Stoll and Gray[8] to account for the differences in UV–VIS spectra of isomeric vinyl-substituted bile pigments.



Scheme 1 *Reagents and conditions:* i, in MeOH, **2** (>0.1 mol dm^{-3}), H_2SO_4 or CSA (0.5 mol dm^{-3}); ii, 20–65 °C; iii, 65 °C; iv, H_2SO_4; v, MeOH



Scheme 2 Two mesomers of the species **2**·H^+

vinyl substituent essentially proceeds without participation of the extended π-electron system of the bilatriene. Thus for biliverdin-XIIIα in which both vinyl groups are located at such unfavourable positions, dimers or methanol adducts are not observed if submitted to analogous acidic conditions as described for compound **2**.

Considering first the reactions at medium acid concentrations, species **2**·H^+ may alternatively be regarded as a vinylogous cation by simple electron shift transferring the positive charge from the pyrrolenine nitrogen to the C-2^2 position (see above and Scheme 2). This potential vinylogous cation undergoes electrophilic reaction with the 2-vinyl group of a second biliverdin entity **2**·H^+ to give species **A** (Scheme 1). This initial step, by which junction between two molecules of **2**·H^+ takes place, is reminiscent of the intramolecular ring closure between the 2- and 18-vinyl groups in biliverdin-IIIα dimethyl ester **1**.[1] However, the further course of the reaction is quite different. While in the case of compound **1** the corresponding carbocation primarily formed experiences addition of a methoxy or hydroxy group with subsequent oxidation, intermediate **A** undergoes an intramolecular reaction by electrophilic attack of the positive charge at C-1′ on the double bond at C-4′. Simultaneously, the positive charge becomes retransferred to the pyrrolenine nitrogen. This two-step mechanism accounting for the formation of compound **4** is more realistic than a concerted, thermally forbidden (2 + 2) cycloaddition.

The thermally promoted, acid-catalysed reaction of the cyclobutane **4** mainly affording compound **5b** can be related to a vinylcyclobutane–cyclohexene-like rearrangement.[11] At the present time it is not clear whether this transformation comprises a concerted [1,3]-sigmatropic process as formulated in Scheme 1 or proceeds through intermediates. Arguments in

favour of concertedness are provided by the high stereoselectivity (90%) and by the indication that the potential intermediate **A** ⟷ **B** is *not* involved in the conversion **4** ⟶ **5** (see below).

Besides the formation of isomers **5a** and **5b** *via* compound **4** there must exist a second route. This follows from the quite different isomer ratio **5a**:**5b** = 2:3 obtained if compound **2** is used as starting material, and from the fact that even prolonged heating in the presence of acids does not change the isomer ratio or lead to interconversion of pure isomers. Clearly, taking into account the mesomeric nature of intermediate **A** ⟷ **B**, attack of the positive charge at C-3 on the C-4′ double bond likewise affords compounds **5**. Therefore, pathway **4** ⟶ **5** does not proceed through intermediate **A** ⟷ **B**.* However, the isomer ratio **5a**:**5b** obtained *via* compound **2** as starting material does not reflect the characteristics of one pathway alone since formation from the cyclobutane **4** proceeds simultaneously.

The mechanisms proposed are consistent with results from deuteriation experiments. If the reactions under consideration are performed with methan[^{2}H]ol–sulfuric [$^2H_2$]acid no deuterium was found in any *C*-position of compound **4**. In isomers **5a** and **5b** deuterium was incorporated at C-5 of the corresponding hydrobiliverdin moieties. However, this finding is a characteristic feature of hydrobiliverdins in general [12] rather than reflecting their modes of formation. In fact, compounds **5** themselves undergo smooth and reversible [^{1}H]–[^{2}H] exchange at C-5 if heated in the presence of methan[^{2}H]ol–sulfuric [$^2H_2$]acid.

At large excess of acid over compound **2** addition of a proton at the C-2[2] vinylic position becomes increasingly important favouring the formation of compounds **6** and **7** and the methoxybiliverdin **3**, too. On performing the reaction in a two-step fashion by treating substrate **2** with conc. sulfuric acid followed by quenching with methanol, compounds **3**, **4** and **5** can no longer be detected. Dimerisation is initiated by intermolecular reaction of the intermediate carbocation **2**·H_2^{2+} with the 2-vinyl group of **2**·H^+ affording intermediate **C**. Elimination of a proton from C-1 and reaction with methanol yields the olefin **6** and its adducts **7a** and **7b**, respectively. Dimers **6**, **7a** and **7b** are interconvertible if subjected to the conditions given above. These reactions are closely related to the acid-catalysed dimerisation of olefins.

Under all conditions employed using acid *and* methanol as reaction medium the methanol adduct **3** is additionally obtained in varying amounts depending upon the concentrations of acid and compound **2**. At medium acid concentration, as used for the conversion **2** ⟶ **4** + **5**, only traces of compound **3** are formed since population of the species **2**·H_2^{2+} is small. Nevertheless one could expect to obtain a (anti-Markovnikov) methanol adduct isomeric with compound **3** by reaction of the potential vinylogous cation **2**·H^+ (Scheme 2) with a methoxy anion. However, if this process occurs at all the tautomeric allyl ether primarily formed would be reversibly cleaved into its components under the conditions employed.

Conclusions

The study presented here supplements and enlarges the knowledge about the subtile chemistry of bile pigments and contributes to a better understanding of the reaction modes of biliverdins. The differences in the ability of the extended π-electron system of bilatrienes to promote electrophilic additions to a vinyl substituent, and the orientation of these reactions are related to the position of the substituent and are due to differences in mesomeric effects. This comprises a more plausible rationale for the vinyl group's reactivities of compound **2** than that given in ref. 13. The anchimeric acceleration claimed therein would not account for the smooth addition of methanol to vinyl groups at C-7(C-13) and C-8(C-12) observed for biliverdin-IXβ, -IXγ and -IXδ,[14] nor explain the orientation of these additions. On the other hand even the intramolecular cyclisations observed for biliverdin-IIIα,[1] -IXγ and -IXδ[14] fit the expectations derived from our concept.

Apart from these more general aspects our investigation permits a convenient access to bichromophoric bilatrienes which would have been difficult to prepare otherwise. Since compound **2** is easily available *via* oxidation of commercial bilirubin-IXα, dimers **4**–**7** require essentially a one-step synthesis which emphasises the utility of the reactions reported here. The bilatriene moieties adopt distinct conformations and are separated by defined but different distances depending on the links between them. In stereoisomers **5a** and **5b** even their relative orientation is mainly fixed. Since bilatrienes in general can be transformed into a variety of other open-chain chromophores which in turn may also be cyclised to porphyrins† and corrins compounds **4**–**7** should be suitable precursors for the investigation of intramolecular electronic interaction and electron transfer between tetrapyrrolic entities over relatively short and adjustable distances.

Experimental

M.p.s were determined with a Kofler–Reichert hot-stage apparatus and are uncorrected. ^{1}H NMR (250 or 400 MHz) and ^{13}C NMR (62.9 MHz; *J*-modulated) spectra were recorded with Bruker instruments (WM 250, AM 400 WB) at 297 K for 10^{-2} mol dm^{-3} solutions in $CDCl_3$ (chromatographed on alumina prior to use), [$^2H_5$]pyridine, [$^2H_4$]methanol and [$^2H_6$]benzene, with $SiMe_4$ as internal reference. *J*-Values are in Hz. Variable-temperature ^{1}H NMR spectra were performed in $CDCl_3$–[$^2H_4$]methanol (9:1) (293 K–213 K) and hexachlorobuta-1,3-diene–[$^2H_6$]dimethyl sulfoxide (1:1) (293 K–373 K). NOE difference spectra were obtained for 5 × 10^{-3} mol dm^{-3} deaerated solutions. 2D-NMR experiments were performed with a Bruker AM 400 WB instrument at 400.1 MHz for ^{1}H and 100.6 MHz for ^{13}C, respectively, using standard Bruker software under the following conditions: s.w., 3200 Hz; pulse width (p.w.), 10 μs; number of fids (n.i.), 256; and data size (zero-filling to) 1024 × 1024 for double-quantum-filtered phase-sensitive COSY, and s.w. (^{1}H), 3200 Hz; s.w. (^{13}C), 16 000 Hz; p.w. (inverse ^{1}H; 90°), 14 μs; p.w. (^{13}C; hard; 90°), 5 μs; p.w. (^{13}C; soft; 90°), 50 μs; n.i., 512; data size (zero-filling to) 1024 × 2048 for proton-detected[16] phase-sensitive ^{1}H–^{13}C shift correlation (with a BIRD-pulse sequence and GARP-1 ^{13}C decoupling during acquisition). Assignments of ^{13}C chemical shifts were further supplemented by comparison with estimated shift values using the 'CSEARCH'-database.[17] Molecular masses were determined by fast-atom bombardment (FABMS) and field desorption (FDMS) mass spectrometry using a Finnigan MAT 900 (glycerol/phosphoric acid; Xe) and a Finnigan MAT 8230 instrument, respectively. UV–VIS spectra were measured with a Perkin-Elmer Lambda 7 spectrometer (1 cm quartz cuvettes), and CD spectra were taken with a Jobin Yvon CD6 dichrograph (accumulative mode, 10 cycles) carrying cylindrical quartz cuvettes (1 cm). Optical rotations (10 cm path length) were obtained with a Perkin-Elmer 241 instrument. All optical measurements were carried out in thermostatted cell compartments (20 ± 1 °C) in spectroscopic-grade chloroform (Lichro-

* Clearly, if the direct transformation **2** ⟶ **5a** + **5b** is regarded as a concerted Diels–Alder addition, intermediate **B** can no longer be excluded for the conversion **4** ⟶ **5**.

† For a porphyrin analogue of compound **6** see ref. 15.

solv, Merck, chromatographed on alumina prior to use). UV–VIS spectra refer to $\sim 2 \times 10^{-5}$ mol dm^{-3} solutions in chloroform. Column chromatography was performed on silica gel (Kieselgel 60, 230–400 mesh, Merck) with mixtures of chloroform and acetone (purified with silica gel prior to use) as eluent. For TLC Kieselgel 60 plates (Merck) were used. R_f-Values compare with the order of elution given for column chromatography. The optically active reagents used showed satisfactory optical rotations: (*R*)-(−)-mandelic acid (Fluka) $[\alpha]_D^{20}$ −142.2 10^{-1} deg cm^2 g^{-1} (*c* 5, water); (1*S*)-(+)-camphor-10-sulfonic acid monohydrate (Merck) $[\alpha]_D^{20}$ +20.5 10^{-1} deg cm^2 g^{-1} (*c* 7, water) and (*S*)-(−)-ethyl lactate (Merck, distilled) $[\alpha]_D^{20}$ −11.1 10^{-1} deg cm^2 g^{-1} (neat). For reactions, methanol and sulfuric acid (95–98%) (both p.A., Merck) were used without further purification. Compounds **2**[18] and **3**[13] were prepared according to standard procedures. The drying agent was Na_2SO_4. After column chromatography the compounds were triturated with CH_2Cl_2 (0.1–0.5 cm^3). After addition of hexane (3–8 cm^3) and sonication (5 min) the mixture was centrifuged and the supernatant was discarded. The dark microcrystalline residue was dried at 40 °C under reduced pressure.

Formation of Compounds **4**, **5a** *and* **5b** *from Compound* **2**: (1R,2R) *and* (1S,2S)-1,2-*Bis*-[8′,12′-*bis*-(2-*methoxycarbonylethyl*)-3′,7′,13′,18′-*tetramethyl*-1′,19′-*dioxo*-17′-*vinyl*-1′,19′,21′,24′-*tetrahydrobilin*-2′-*yl*]*cyclobutane* **4** *and* [(3R,2^{4}S) *and* (3S,2^{4}R)]- *and* [(3R,2^{4}R) *and* (3S,2^{4}S)]-*Dimethyl* 2^4-[8′,12′-*Bis*-(2-*methoxycarbonylethyl*)-3′,7′,13′,18′-*tetramethyl*-1′,19′-*dioxo*-17′-*vinyl*-1′,19′,21′,24′-*tetrahydrobilin*-2′-*yl*]-3,7,13,18-*tetramethyl*-1,19-*dioxo*-17-*vinyl*-1,2^2,2^3,2^4,3,19,21,24-*octahydrobenzo*[b]*bilin*-8,12-*dipropionate** **5a** *and* **5b**.—*Procedure A*. A solution of compound **2** (350 mg, 0.57 mmol) and (1*S*)-(+)-camphor-10-sulfonic acid monohydrate† (710 mg, 2.85 mmol) in methanol (4 cm^3) was stored at room temperature for 30 days. Then water (50 cm^3) was added and the solution was neutralised ($NaHCO_3$). This mixture was stripped with chloroform (2 × 60 cm^3), and the combined extracts were washed with water and dried. After evaporation of the solvent under reduced pressure the residue was subjected to column chromatography (column 4 × 40 cm;‡ chloroform–acetone with a gradient from 88:12 to 80:20) to afford starting material **2** (240 mg recovery), compound **4** (28 mg, 26%), and isomers **5a** and **5b** (4 and 6 mg, respectively, 9%) in that order; total 35% based on consumed substrate **2**.

Procedure B. A mixture of compound **2** (300 mg, 0.49 mmol), methanol (2 cm^3) and sulfuric acid (0.1 cm^3, 1.8 mmol) was stirred at 65 °C for 24 h. Work-up and chromatography as described for procedure A afforded starting material **2** (120 mg recovery), compound **4** (28 mg, 16%) and isomers **5a** and **5b** (24 and 33 mg, respectively, 32%) in that order; total 48% based on starting material consumed.

Compound **4** had m.p. 185–190 °C (decomp.) (Found: C, 68.4; H, 6.1; N, 9.2. $C_{70}H_{76}N_8O_{12}$ requires C, 68.8; H, 6.3; N, 9.2%); *m*/*z* (FABMS) 1221.5 ($M^+ + H$); λ_{max}($CHCl_3$)/nm 641 (ε 28 500), 375 (90 000) and 280 (35 900) (*f* 2.14),§ see also Fig. 1; δ_H([$^2H_5$]pyridine; 250 MHz) 11.63 and 11.20 (2 H × 2, s br, 21′-H, 24′-H), 7.04 (2 H, s, 10′-H), 6.79, 5.71 and 5.60 (2 H × 3, XMA, J_{XM} 17.5, J_{XA} 11.5, J_{MA} 1.5, 17′-Vn), 5.99 (2 H, s, 15′-H), 5.73 (2 H, s, 5′-H), 4.51 (2 H, m, 1- and 2-H), 3.61 (6 H, s, CO_2Me), 3.60 (6 H, s, CO_2Me), 3.12 and 2.40 (2 H × 2, m, 3-H and 4-H), 2.95 (8 H, m, 8′- and 12′-CH_2), 2.64 (8 H, m, CH_2CO_2), 2.17 (6 H, s, 18′-Me), 2.15 (6 H, s, 3′-Me), 1.94 (6 H, s, 13′-Me) and 1.91 (6 H, s, 7′-Me); δ_C([$^2H_5$]pyridine; 62.9 MHz) 173.72− and 173.08− (*C*O_2Me and CO−1′ and CO−19′), 127.32+ (17′-*C*H=CH_2), 122.18− (17′-CH=*C*H_2), 115.31+ (CH-10′), 97.34+ (CH-15′), 96.35+ (CH-5′), 51.46+ (CO_2*Me*), 36.58+(CH-1 and -2), 35.54− (*C*H_2CO_2), 23.83− (CH_2-3, CH_2-4), 20.03− (8′-and 12′-CH_2), 10.14+ (18′-Me), 9.61+ (3′-Me) and 9.25+ (7′- and 13′-Me); carbons not listed have not been assigned; for ^{1}H and ^{13}C NMR data in $CDCl_3$ see Tables 1–3.

Compound **5a** had m.p. 190–200 °C (decomp.) (Found: C, 68.7; H, 6.6; N, 9.0. $C_{70}H_{76}N_8O_{12}$ requires C, 68.8; H, 6.3; N, 9.2%); *m*/*z* (FABMS) 1221.3 ($M^+ + H$); λ_{max}($CHCl_3$)/nm 626 (ε 28 800), 372 (80 600) and 276 (41 700); for ^{1}H and ^{13}C NMR data see Tables 1–3 (non-systematic numbering scheme applies).

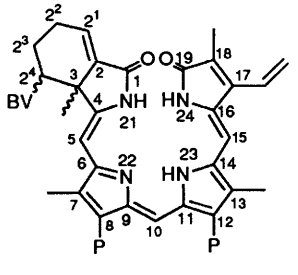
Compound **5b** had m.p. 260–270 °C (decomp.) (Found: C, 68.5; H, 6.3; N, 8.9%); *m*/*z* (FABMS) (1221.3 ($M^+ + H$); λ_{max} ($CHCl_3$)/nm 619 (ε 28 700), 368 (76 900) and 275 (40 600); for ^{1}H and ^{13}C NMR data see Tables 1–3 (non-systematic numbering scheme applies)

Deuteriation Experiments.—If in procedures A and B deuteriated reagents (sulfuric [$^2H_2$]acid and methan[^{2}H]ol), were used no deuterium was incorporated in any C-position of product **4** (FABMS; ^{1}H NMR). Compounds **5** showed [^{1}H]–[^{2}H] exchange only at C-5 (FABMS; ^{1}H NMR). Treatment of pure and undeuteriated isomers **5** under the same conditions led to the same results within 1 h.

Asymmetric Synthesis of the Chiral Cyclobutane (+)-**4**.—A mixture of compound **2** (100 mg, 0.16 mmol), sulfuric acid (0.1 cm^3, 1.8 mmol) and (*S*)-(−)-ethyl lactate (1.9 cm^3) was stirred at room temperature until the solution became homogeneous (24 h) and was then kept for 24 days in the dark. After usual work-up (see procedure A) the remaining ethyl lactate was removed at ambient temperature under reduced pressure. The residue was then treated with methanol–sulfuric acid (5% v/v) (10 cm^3) for 2 h. Work-up and chromatography as described for procedure A furnished starting material **2** (60 mg recovery), the methoxy adduct **3** (4 mg; identical with that prepared *via* bilirubin as revealed by m.p., ^{1}H NMR and UV–VIS spectrum)[13,19] and compound (+)-**4** (3 mg, 8% based on consumed **2**); $[\alpha]_{436}^{20}$ +300 ± 100 10^{-1} deg cm^2 g^{-1} (*c* 0.002, $CHCl_3$); CD λ_{max}($CHCl_3$)/nm 645 (Δε −2.3) and 372 (+3.2); in all other respects (m.p., NMR, MS and UV–VIS spectra) optically active (+)-**4** agreed with racemic **4**. The ee (14%) was determined by addition of (*R*)-(−)-mandelic acid (2 mol equiv.) to a solution of compound (+)-**4** in $CDCl_3$–[$^2H_6$]benzene (1:1) and integration of the well resolved 15′-H signals in the ^{1}H NMR spectrum. The CD of the long wavelength band of enantiomerically pure (+)-**4** centred around λ 645 nm thus amounts to Δε −16.4.

Formation of Isomers **5** *from Substrate* **4**.—A solution of compound **4** (14 mg, 0.011 mmol) in methanol (0.5 cm^3) containing sulfuric acid (0.025 cm^3, 0.45 mmol) was maintained

* Systematic numbering



† Note that CSA is used only to increase the solubility of compound **2**. Its optical activity is irrelevant for the reaction *per se*. The products formed (**4**, **5a** and **5b**) are throughout racemic.

‡ If the diameter is smaller, products may occasionally precipitate during elution.

§ For determination of *f*, the quotient of dipole strengths of the first UV- and the VIS-band, integration was performed from λ 480 to 330 nm (cutoff) and from λ 800 to 500 nm, respectively.

at 65 °C for 7 h. Work-up and column chromatography as described for procedure A afforded starting material **4** (5 mg recovery) and products **5a** (0.4 mg, 4%) and **5b** (4.4 mg, 49%), total 53% based on consumed **4**, identical in all respects with the compounds obtained *via* compound **2** (m.p.; FABMS; NMR and UV–VIS spectra). If deuteriated reagents were used deuterium was only incorporated at C-5 of the products **5a** and **5b** (FABMS; ^{1}H NMR) as found for the direct conversion 2 ⟶ **4**, **5a** and **5b** (see above).

Formation of Compounds **6**, **7a** *and* **7b** *from Substrate* **2**: (3R *and* 3S)-1,3-*Bis*-[8′,12′-*bis*-(2-*methoxycarbonylethyl*)-3′,7′,13′,18′-*tetramethyl*-1′,19′-*dioxo*-17′-*vinyl*-1′,19′,21′,24′-*tetrahydrobilin*-2′-*yl*]*but*-1(E)-*ene* **6**, *and* [(1R,3R) *and* (1S,3S)] *and* [(1R,3S) *and* (1S,3R)]-1,3-Bis[8′,12′-bis(2-methoxycarbonylethyl)-3′,7′,13′,18′-*tetramethyl*-1′,19′-*dioxo*-17′-*vinyl*-1′,19′,21′,24′-*tetrahydrobilin*-2′-*yl*]-1-*methoxybutane* **7a** *and* **7b** (*or vice versa*).—A mixture of compound **2** (90 mg, 0.15 mmol) and sulfuric acid (1.5 cm^3) was sonicated for 5 min at room temperature and then poured into cold (−20 °C, procedure C) and hot (65 °C, procedure D) methanol (60 cm^3), respectively (**CAUTION**!). After 10 min water (200 cm^3) was added and the solution was neutralised (pH 7.5, $NaHCO_3$). The mixture was stripped with chloroform (3 × 50 cm^3), and the combined extracts were washed with water (2 × 50 cm^3) and dried. The solvent was evaporated off under reduced pressure and the residue was subjected to column chromatography (column 4 × 30 cm,* with chloroform–acetone gradient from 88:12 to 80:20; after elution of compound **6** 2% v/v methanol was added).

Procedure C gave compounds **6** (5 mg, 6%), **7a** and **7b** (33 and 32 mg, respectively, 70%) in that order; overall yield 76%.

Procedure D gave compounds **6** (37 mg, 41%), **7a** and **7b** (15 and 12 mg, respectively, 29%); overall yield 70%.

Compound **6** had m.p. 188–190 °C (decomp.) (Found: C, 68.8; H, 6.4; N, 9.0. $C_{70}H_{76}N_8O_{12}$ requires C, 68.8; H, 6.3; N, 9.2%); *m/z* (FDMS) 1220 (M^+); $\lambda_{max}(CHCl_3)$/nm 646 (ε 29 600), 381 (99 600), 316 (43 600) and 282 (37 700) (*f* 2.40)† see also Fig. 4; for ^{1}H and ^{13}C NMR data see Tables 1–3.

Compound **7a** had no m.p. up to 300 °C (Found: C, 67.7; H, 6.4; N, 8.6. $C_{71}H_{80}N_8O_{13}$ requires C, 68.0; H, 6.4; N, 8.9%); *m/z* (FDMS) 1252 (M^+); $\lambda_{max}(CHCl_3)$/nm 639 (ε 28 300), 375 (89 300) and 276 (36 600) (*f* 2.16),† see also Fig. 1; for ^{1}H and ^{13}C NMR data see Tables 1–3.

Compound **7b** had no m.p. up to 300 °C (Found: C, 67.9; H, 6.3; N, 8.8%); *m/z* (FDMS) 1252 (M^+); $\lambda_{max}(CHCl_3)$/nm 632 (ε 28 500), 371 (83 300) and 275 (35 000) (*f* 2.01),† see also Fig. 1; for ^{1}H and ^{13}C NMR data see Tables 1–3.

* If the diameter is smaller, products may occasionally precipitate during elution.

† For determination of *f*, the quotient of dipole strengths of the first UV- and the VIS-band, integration was performed from λ 480 to 330 nm (cutoff) and from λ 800 to 500 nm, respectively.

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