Chemical Total Synthesis of (+)-(2*R*,16*R*)- and (+)-(2*S*,16*R*)-Phycoerythrobilin Dimethyl Ester[1]

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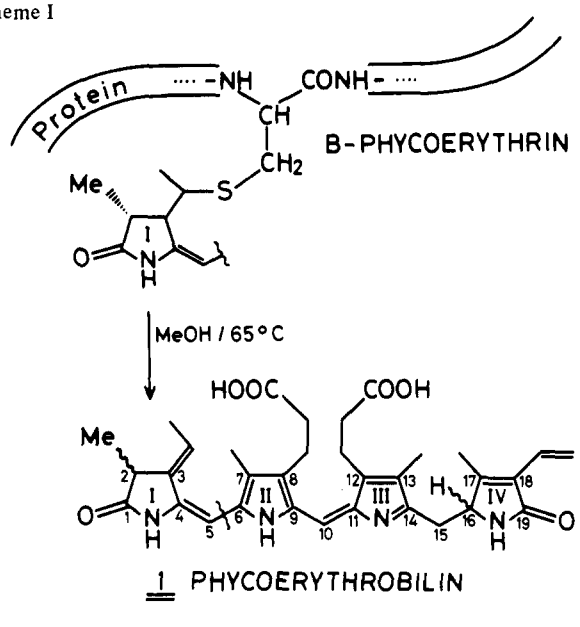
Abstract: The title compounds, two bile pigment-like products isolated from the photosynthetically active chromoproteins of the blue-green and red algae, have been synthesized chemically by condensation of racemic **20** with optically active **19** and subsequent chromatographic separation of the diastereomeric reaction products. Decisive for the resolution of the racemic precursors of **19** was the use of the α-methylfenchyl ester group which behaves chemically equivalent to a *tert*-butyl group throughout the several steps of the convergent synthesis. The absolute configuration of **19** has been determined by condensing it with (+)-(*R*)-5′-hydroxycarbonylneobilirubinic acid (**22**) to yield (+)-(2*R*,16*R*)-[18-vinyl]mesourobilin IXα dimethyl ester (**23**), whose configuration has been established on the basis of Moscowitz's model for optically active urobilinoids. The absolute configurations of both diastereomeric phycoerythrobilin dimethyl esters **21a** and **21b** at C-2 and C-16 were inferred from that of their precursor **19** and of the (*E*)-ethylidene methyl succinimides which were obtained by oxidative degradation of each bile pigment, respectively. From the comparison of the synthetic phycoerythrobilins with the purple pigment isolated from *C*-phycoerythrin by treatment with boiling methanol, it follows that the chromophore of the native chromoprotein has the *R* configuration at both C-2 and C-16.

Phycoerythrobilin (**1**) is the purple pigment released by boiling methanol from the photosynthetically active chromoproteins *R*-, *B*-, and *C*-phycoerythrin which occur in most red and some blue-green algae.[2,3] At the present time, it is well established that the protein moiety of both *B*-[4] and *C*-phycoerythrin[5] (as probably also of other biliproteins[6]) is covalently bound to the prosthetic group, whereby one of the covalent bonds connects the sulfur atom of a cysteine molecule with the ethyl group on ring I[4] (cf. Scheme I). On treatment with boiling methanol, an elimination reaction occurs which involves cleavage of the thioether bond, and the exocyclic double bond of the ethylidene group of the released phycoerythrobilin is formed. The structure of the latter has been elucidated by means of both spectroscopic[7,8] and degradation studies.[9,10] Hitherto, however, no sound assignments have been made concerning the stereochemistry of phycoerythrobilin. As chromic acid oxidation of phycoerythrobilin yields only (*E*)--ethylidenemethylsuccinimide among the degradation products,[10] the double bond of the ethylidene group attached to C-3 in **1** has most likely the *E* configuration. Under the same conditions, however, a 2:7 mixture of the *Z* and *E* isomers of ethylidenemethylsuccinimide has been isolated from *B*-phycoerythrin,[11] thus supporting that the ethylidene group of phycoerythrobilin is not present in the native biliprotein. On the other hand, according to the chiroptical properties of an urobilinoid pigment which was obtained on reoxidation of the leuco derivative formed by catalytic hydrogenation of phycoerythrobilin, the *R* configuration has been ascribed to the asymmetric C-16 atom of the latter.[12]

Scheme I



Now we report a convergent total synthesis of (+)-phycoerythrobilin dimethyl ester (**21a**) which not only confirms the structure assigned to this compound but also enables the unequivocal assignment of the absolute configurations at both chirality centers C-2 and C-16 as well as the configuration of the ethylidene double bond at C-3. The key intermediates for the synthesis of **21a**, namely, the 5(1*H*)-pyrromethenone derivative **20** and the 5(2*H*)-dipyrrylmethanone aldehyde *rac*-**19**, were made available earlier in the course of our syntheses of phycocyanobilin dimethyl ester[13,14] and of [18-vinyl]-mesourobilin IXα dimethyl ester,[15,16] respectively. However, in order to synthesize optically active phycoerythrobilin dimethyl ester, at least one of the reactants had to be prepared as a single enantiomer. Owing to the fact that 5′-hydroxycarbonylisoneobilirubinic acid (*rac*-**15**, ethyl instead of vinyl) could be resolved in its antipodes by fractional crystallization of their corresponding salts with optical active bases,[17] we intended to carry out a separation of the racemic mixture of **15** by the same procedure. However, our attempts to liberate the carboxylic acid group at C-5′ by alkaline hydrolysis of the dimethyl ester *rac*-**16**[18] failed; the reaction product was 5′-hydroxycarbonylisoneoxanthobilirubinic acid (**26**), which was probably formed by migration of the vinylic double bond of **15** into the methylene bridge. On the other hand, cleavage of isobutene from the *tert*-butyl ester *rac*-**17**[18] by means of cold trifluoroacetic acid was accompanied by decarboxylation, thus yielding the corresponding methyl (±)-[4-vinyl]isoenobilirubinate (*rac*-**18**)[16] instead of the desired 5′-hydroxycarbonyl derivative. As the carboxylic acid group at C-5′ appears to be indispensable, within this class of compounds, in order to obtain diastereomeric salts with optically active bases capable of being separated by fractional crystallization,[17] we did not attempt to use the conformationally less fixed propionic acid residue of **18** for this purpose. A more promising route to optically active **19** seemed to be the preparation of the ester **14** as an intermediate. Of course, the ester group of **14** should behave like a *tert*-butyl ester whose chemical properties proved to be particularly propitious throughout our earlier work on bile

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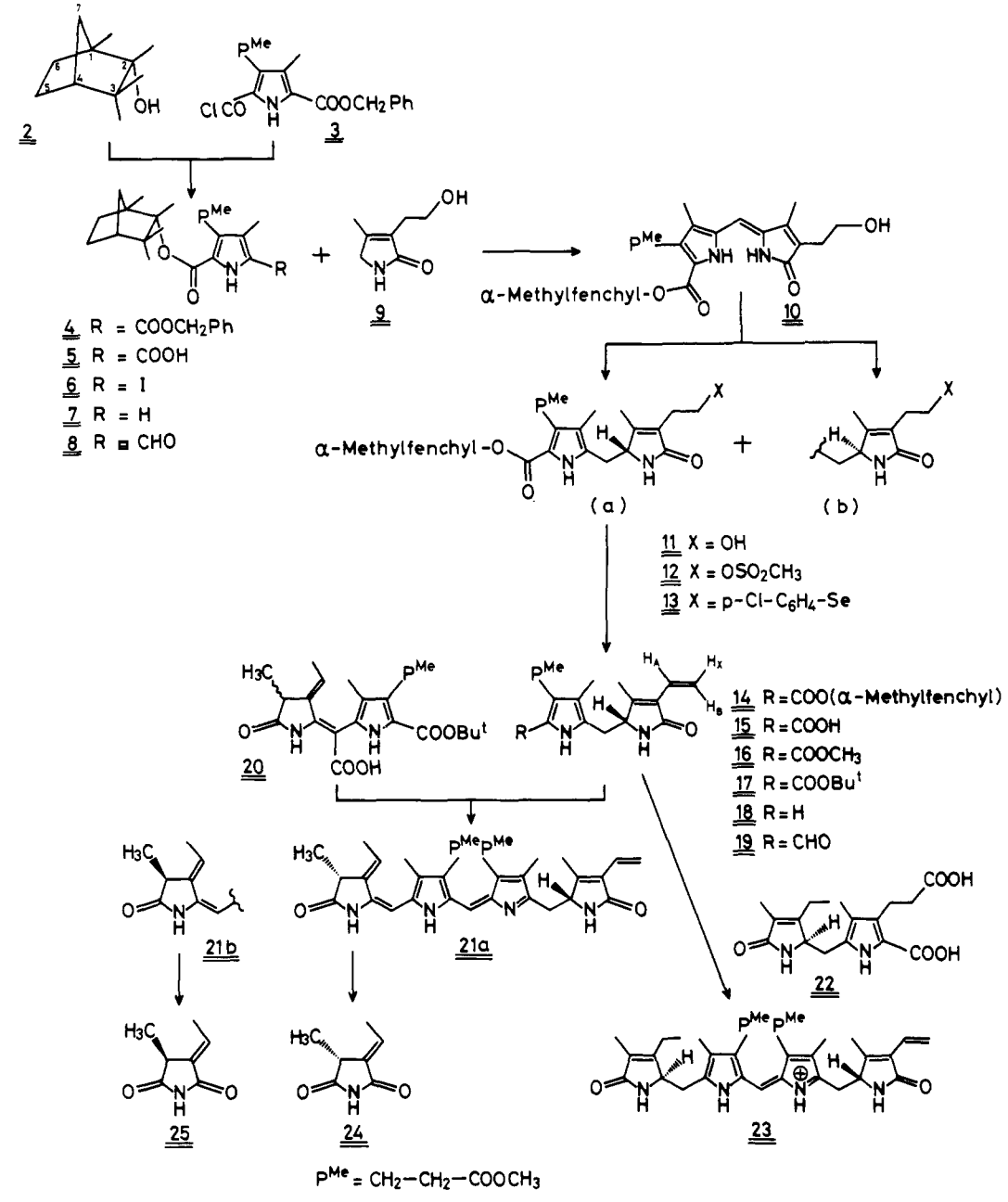

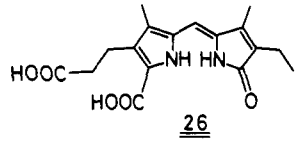

pigments of the phycobilin type. On the other hand, the chiral ester residue should enable the resolution of some of the pairs of diastereomeric 5(2*H*)-dipyrrylmethanones **11–14** obtained after hydrogenation of the exocyclic double bond of **10.** Actually, both expectations proved to be correct.

As one of the starting materials for the synthesis of **14** we prepared optically active α-methylfenchyl alcohol by reaction of commercially available[19] (+)-fenchone ($[\alpha]^{25}_D$ +70.0° in methanol) with methylmagnesium iodide according to the procedure given in ref 20. The main product of the reaction is, presumably, the endo isomer **2.**[21] Reaction of **2** with the already known pyrrolecarboxylic acid chloride **3**[22] yields the corresponding α-methylfenchyl ester **4** ($[\alpha]^{20}_D$ −19.5°) which, after hydrogenolysis of the benzyl ester group, was transformed into the α-unsubstituted pyrrole derivative **7** by decarboxylation of the obtained monocarboxylic acid **5** via iodopyrrole **6.** Vilsmeier formylation of **7** leads to the aldehyde **8** which was condensed, in the presence of base, with the previously described[15] 3-pyrrolin-2-one derivative **9** yielding the 5(1*H*)-pyrromethenone **10.** The exocyclic double bond of the latter was readily hydrogenated on palladized charcoal affording a mixture of two diastereomeric 5(2*H*)-dipyrrylmethanones **11a** and **11b.** As the separation of the two diastereomers failed, both together were transformed into a mixture of the corresponding mesylates **12a** and **12b** which was subsequently treated with *p*-chlorophenyl selenide. The formed selenides could now be resolved by fractional crystallization yielding crystalline **13a** and its diastereomer **13b** which was obtained as an oil. The pure compound **13a** ($[\alpha]^{25}_D$ +56.8°) was transformed into the corresponding selenide oxide which decomposed at room temperature yielding the desired vinyl compound **14.**

As expected, the α-methylfenchyl ester group of **14** could be transformed directly into the formyl group of **19** by reaction with trimethyl orthoformate in trifluoroacetic acid. This behavior parallels the usual reactivity of *tert*-butyl pyrrolecarboxylates which can be converted into the corresponding aldehydes under the same conditions.[23]

In order to elucidate the absolute configuration of **19,** at C-2,

we considered first the possibility of transforming **14** into optically active methyl isoneobilirubinate (**18,** ethyl instead of vinyl) whose absolute configuration could be inferred from the data published in ref 17 and 24. Such a transformation would involve selective hydrogenation of the vinyl group of **14** and subsequent cleavage of the substituent at C-5′. Taking into account, however, that the absolute configuration of isoneobilirubinic had to be assigned indirectly by comparison of the rotatory powers of (+)-mesourobilin IIIα, synthesized therefrom,[17] and that of natural (−)-stercobilin[24] assuming the validity of Moscowitz's model for optically active urobilinoids (see below), we decided to use a more straightforward way of determining the absolute configuration of **14.** Thus, the corresponding optically active aldehyde **19** was condensed with (+)-5′-hydroxycarbonylneobilirubinic acid (**22,** one of the precursors of mesourobilin IXα[17] whose absolute configuration has been assigned as 4*R*,16*R* on the same grounds mentioned above for its isomer[24]) yielding, after treatment with diazomethane, [18-vinyl]mesourobilin IXα dimethyl ester (**23**) of $[\alpha]^{25}_{D}$ +3600°. The reaction product proved to be identical with a sample of the same compound which had been synthesized for the first time in our laboratories by an alternative route.[15] According to Moscowitz's model,[25] the high optical rotation of the urobilins is associated with the occurrence of helix-shaped molecules fixed in one particular conformation by intramolecular hydrogen bonding, thus forming inherent dissymmetric pyrromethene chromophores. Moreover, such helical conformers are obtained only if the absolute configurations at the asymmetric centers C-4 and C-16 are the *same,* i.e. *R,R* or *S,S.* In the case of urobilin **23,** the above-mentioned premises lead to the conclusion that if the absolute configuration of (+)-5′-hydroxycarbonylneobilirubinic acid (**22**) is assumed to be *R,* the absolute configuration of **19** must be *R* also.

Aldehyde **19** was then condensed with the 3,4-dihydro-5(1*H*)-pyrromethenone derivative **20**[14] in the presence of trifluoroacetic acid and methanol to yield a mixture of two diastereomeric phycoerythrobilin dimethyl esters (**21a** and **21b**) with opposite configurations at C-2. Both components could be separated by HP-thin layer chromatography on silica gel whereby the R_f value of the *faster* migrating compound **21a** agrees with that of the *main* product obtained from *C*-phycoerythrin by treatment with boiling methanol. However, depending on the conditions employed for the denaturation of the chromoprotein as well as the subsequent cleavage of the chromophore, fluctuating amounts (up to 40%) of a by-product are obtained[26] which proved to be identical with **21b.** Therefore, some epimerization at C-2 occurs during isolation of phycoerythrobilin dimethyl ester from natural sources.

The analytical and spectroscopical data of **21a,** including melting point and R_f value as well as IR, UV/vis, and ^{1}H NMR spectra, agree with those of an authentic sample of phycoerythrobilin dimethyl ester. Furthermore, all spectroscopic data of **21a** and its R_f value are identical with those of the racemic compound synthesized earlier in our laboratory by an independent route.[27] Of course, none of the investigated analytical data, with the exception of the chromatographic behavior and the melting points, enables us to differentiate between the two diastereoisomers **21a** and **21b.** As expected, the chiroptical properties of both epimers are also very similar, since the main contribution to the chirality of the molecule is provided by the shape-determining position C-16 (see above). Nevertheless, slight differences in the short-wave range of the CD spectra of **21a** and **21b** confirm the identity of the former with "natural" phycoerythrobilin dimethyl ester (cf. Figure 1).

The absolute configuration of **21a** at C-16 is inferred from that of its precursor **19.** On the other hand, the absolute configuration of **21a** at C-2 was determined by chromic acid oxi-

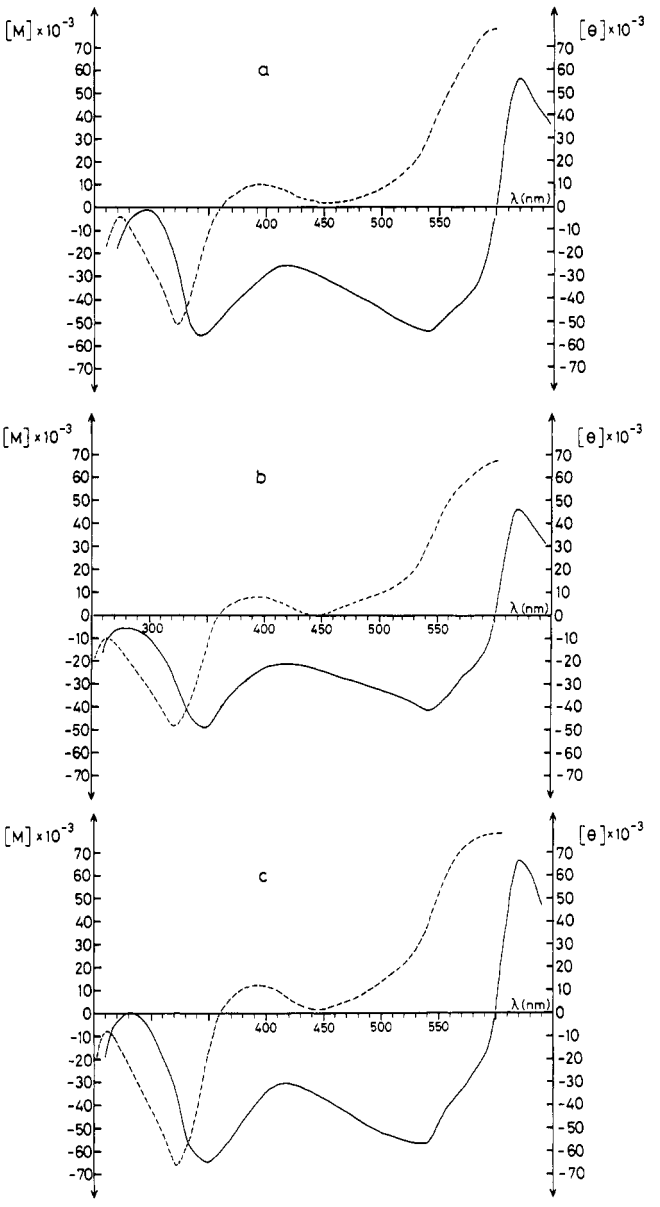

Figure 1. ORD (—) and CD spectra (- - -) of (a) synthetic (+)-(2*R*,16*R*)-phycoerythrobilin dimethyl ester (**21a**), (b) synthetic (+)-(2*S*16*R*)-phycoerythrobilin dimethyl ester (**21b**), and (c) phycoerythrobilin dimethyl ester from *C*-phycoerythrin (in CH_2Cl_2).

dation of the pigment which yielded levorotatory (i.e., *R*-configurated[28]) (*E*)-ethylidenemethylsuccinimide among the degradation products. Accordingly, the corresponding imide isolated from **21b** was dextrorotatory. As the ^{1}H NMR spectra of **21a** and **21b** show well-resolved multiplets in the ranges of absorption of the protons of the ethylidene group whose coupling constants are similar to that of (*E*)-2-ethylidene-3-methylsuccinimide (cf. Experimental Section), it seems likely that the exocyclic double bond at C-2 of both **21a** and **21b** has the *E* configuration. This assumption is corroborated by the oxidative degradation of both synthetic products, whereby only (*E*)-2-ethylidene-3-methylsuccinimide could be detected.[29]

The results reported here not only confirm unequivocally the structure proposed for phycoerythrobilin dimethyl ester but also enable us to conclude that the chromophore of native *C*-phycoerythin (and probably also of the other phycoerythrins) has the *R* configuration at both C-2 and C-16. As two chirality centers of the protein-bound chromophore (C-3 and C-3^1) are lost during cleavage of the protein moiety, the stereochemistry of the phycoerythrins at these positions remains still to be determined unequivocally. On the basis of experi-

ments carried out with substituted succinimides as model compounds, it has been anticipated that both asymmetric C-3 and C-3[1] atoms in *C*-phycoerythin have the *R* configuration.[30]

Experimental Section

Melting points were determined with a Kofler hot stage melting point apparatus (Reichert) and are uncorrected. UV and visible spectra were recorded on a Leitz-Unicam SP 800 B spectrophotometer using methanol solutions unless otherwise specified. The CD spectra were taken with a Roussel-Jouan Dichrographe II and the ORD spectra with a Cary recording spectropolarimeter, Model 60. Infrared spectra (IR) were run on a Perkin-Elmer Model 157 G spectrometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on Varian Associates Model XL 100 and Bruker Model HFX-90 instruments using deuteriochloroform solutions. Chemical shifts (δ) are expressed in parts per million downfield from internal tetramethylsilane and coupling constants (J values) in hertz. Spin multiplicities are indicated by symbols s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Mass spectral (MS) data were obtained at an ionizing voltage of 70 eV on Varian-MAT Model CH 4 and on AEI MS 9 mass spectrometers. The assignments of particular peaks are made on the basis of plausible fragmentation mechanisms which are compatible with the structure of the molecules taken into consideration (cf. ref 31). They have not been verified, however, either by high-resolution mass spectrometry or by means of isotope-labeled derivatives. Elemental analyses were performed by I. Beetz Microanalytical Laboratories, D-8640 Kronach. Preparative layer chromatography of colorless products or pigments made use of 2-mm thick plates measuring 100 × 20 cm precoated with silica gel $PF_{254+366}$ or silica gel H (both from E. Merck, Darmstadt), respectively. R_f values were determined on HPTLC plates (E. Merck).

(−)-4-(Methoxycarbonylethyl)-5-α-methylfenchyloxycarbonyl-3-methylpyrrole-2-carboxylic Acid (5). 5-Benzyloxycarbonyl-3-(2-methyoxycarbonylethyl)-4-methylpyrrole-2-carboxylic acid[32] (6.9 g) was converted into the corresponding acid chloride using thionyl chloride (7 mL) and then treated with (+)-α-methylfenchol[20] [6.0 g, $[\alpha]^{25}_D$ +5.4° (5.7 g/L in methanol)[33]] and *N,N*-dimethylaniline (15 mL) according to the procedure described in ref 22 for the synthesis of *tert*-butyl 5-benzyloxycarbonyl-3-(2-methoxycarbonylethyl)-4-methylpyrrole-2-carboxylate. The crude product **4** in tetrahydrofuran (250 mL) was hydrogenated at 20 °C and 760 Torr on 10% palladized charcoal and the crystalline residue which was obtained after evaporation of the solvent was washed repeatedly with *n*-hexane. Recrystallization from methylene chloride/*n*-hexane yielded 3.1 g (38% overall) of the monocarboxylic acid **5**: mp 110 °C; $[\alpha]^{20}_D$-19.5° (2.6 g/L in methanol); ORD (2.6 g/L in methanol) λ [M] 600 (−62), 305 (−1340), 295 (−1020), 288 (−1180), 272 nm (−190°); IR (KBr) 3290, 2860, 1700, 1670, 1640, 1450, 1340, 1270, 1200 cm^{-1}; ^{1}H NMR (90 MHz) δ 1.00 (3 H), 1.12 (6 H) (each s, 1′- and 3′-CH_3), 1.4–2.1 (m, 7 H, fenchyl-CH_2 and 4′-H), 1.56 (s, 2′-CH_3), 2.33 (s, 4-CH_3), 2.51 (t, J = 8 Hz, α-CH_2 of propionic ester), 3.09 (t, J = 8 Hz, β-CH_2 of propionic ester), 3.67 (s, OCH_3), 9.38 (br s, NH), 10.29 (br s, COOH); MS *m/e* 405 (M^+), 255 (M^+ − 1-methylcamphene, 211 (255 − CO_2).

(−)-α-Methylfenchyl 5-Iodo-3-(2-methoxycarbonylethyl)-4-methylpyrrole-2-carboxylate (6). A stirred solution of the above monocarboxylic acid (3.0 g) in methanol (100 mL) containing a saturated aqueous solution of sodium bicarbonate (20 mL) was treated dropwise with a methanolic solution of iodine (1.9 g in 30 mL). After addition was complete, the mixture was stirred for a further 30 min at room temperature and then boiled until it became pale yellow. The solution was diluted with water (300 mL) and extracted repeatedly with methylene chloride. The combined organic phases were filtered through methylene chloride soaked filter paper and the solvent was evaporated under reduced pressure. The remaining oily residue (2.6 g, 72%) was used without further purification in the next reaction: IR (neat) 3260, 2920, 1740, 1670, 1560, 1440, 1390 cm^{-1}; ^{1}H NMR (90 MHz) 0.98, 1.09, and 1.10 (each s, each 3 H, 1′- and 3′-CH_3), 1.4–2.1 (m, 7 H, fenchyl-CH_2 and 4′-H), 1.51 (s, 2′-CH_3), 1.99 (s, 4-CH_3), 2.40 (t, J = 8 Hz, α-CH_2 of propionic ester), 3.01 (t, J = 8 Hz, β-CH_2 of propionic ester), 3.58 (s, OCH_3), 9.00 (br s, NH); MS *m/e* (rel intensity) 487 (16) M^+, 337 (100) M^+ − 1-methylcamphene, 320 (35), 293 (25) 337 − CO_2, 287 (58), 246 (22), 167 (27).

(−)-α-Methylfenchyl 3-(2-Methoxycarbonylethyl)-4-methylpyrrole-2-carboxylate (7). Magnesium oxide (5 g) was added to a solution of the foregoing iodopyrrole (2.5 g) in methanol (100 mL), and the mixture was hydrogenated (40 atm) for 12 h at room temperature on 10% palladized charcoal. After filtration, the solvent was evaporated under reduced pressure and the oily residue was chromatographed on silica gel eluting with methylene chloride. Crystallization from *n*-hexane yielded pure **7** (1.4 g, 76%): mp 103–105 °C; $[\alpha]^{25}_D$ −28.7° (7.2 g/L in methanol); ORD (7.2 g/L in methanol) λ [M] 600 (−101), 350 (−440), 300 nm (−970°); IR (KBr) 3260, 2870, 1740, 1650, 1440, 1370, 1350, 1280 cm^{-1}; ^{1}H NMR (90 MHz) δ 1.02, 1.09, and 1.14 (each s, each 3 H, 1′- and 3′-CH_3), 1.4–2.1 (m, 7 H, fenchyl-CH_2 and 4′-H), 1.56 (s, 2′-CH_3), 2.04 (s, 4-CH_3), 2.53 (t, J = 8 Hz, α-CH_2 of propionic ester), 3.11 (t, J = 8 Hz, β-CH_2 of propionic ester), 3.67 (s, OCH_3), 6.63 (d, J = 2.5 Hz, 5-H), 8.78 (br s, NH); MS *m/e* (rel intensity) 361 (13) M^+, 211 (41) M^+-1-methylcamphene, 194 (41), 167 (51) 211 − CO_2, 152 (100).

Anal. ($C_{21}H_{31}NO_4$) C, H, N.

(−)-α-Methylfenchyl 5-Formyl-3-(2-methoxycarbonylethyl)-4-methylpyrrole-2-carboxylate (8). The above α-free pyrrole (1.0 g) was formylated according to the method described elsewhere[34] for the corresponding 2-*tert*-butyl ester using a solution of Vilsmeier's reagent which was prepared by reaction of phosphorus oxychloride (0.6 mL) with dimethylformamide (4 mL). The yield of crystalline **8** amounted to 0.9 g (83%): $[\alpha]^{25}D$ −6.7° (32 g/L in methanol); ORD (32 g/L in methanol) λ [M] 600 (−25), 400 (−88), 310 nm (−450°); IR (neat) 3250, 1700, 1650, 1440, 1250 cm^{-1}; ^{1}H NMR (90 MHz) 1.00 (3 H) and 1.13 (6 H) (each s, 1′- and 3′-CH_3), 1.4–2.1 (m, 7 H, fenchyl-CH_2 and 4′-H), 1.56 (s, 2′-CH_3), 2.33 (s, 4-CH_3), 2.44 (t, J = 8 Hz, α-CH_2 of propionic ester), 2.98 (t, J = 8 Hz, β-CH_2 of propionic ester), 3.60 (s, OCH_3), 9.40 (br s, NH), 9.71 (s, CHO); MS *m/e* (rel intensity) 389 (17) M^+, 239 (29) M^+ − 1-methylcamphene, 227 (23), 180 (66), 107 (86), 57 (83), 41 (100).

(−)-α-Methylfenchyl 3,3′-Dimethyl-4-(2-hydroxyethyl)-4′-(2-methoxycarbonylethyl)-5(1*H*)-2,2′-pyrromethenone-5′-carboxylate (10). A solution of the foregoing aldehyde (1 g) and 3-(2-hydroxyethyl)-4-methyl-3-pyrrolin-2-one[15] (360 mg) in a mixture of methanol (30 mL) and 4 N aqueous KOH (30 mL) was stirred for 14 h at room temperature. The crude product which was isolated according to the procedure described in ref 15 for the corresponding 5′-*tert*-butyl ester was purified by chromatography on silica gel eluting with methylene chloride/methanol (96:4) to yield 1 g (75%) of **10**: mp 160 °C (from methylene chloride/*n*-hexane): $[\alpha]^{25}_D$ −108.8° (2.6 g/L in methanol); ORD (87 mg/L in methanol) λ [M] 600 (−540), 432 (−3000), 374 nm (0°); IR (KBr) 3330, 2900, 1740, 1670, 1440, 1280 cm^{-1}; ^{1}H NMR (90 MHz) δ 0.99 (3 H) and 1.07 (6 H) (each s, 1″- and 3″-CH_3), 1.3–1.8 (m, 7 H, fenchyl-CH_2 and 4″-H), 1.50 (s, 2″-CH_3), 2.11 (s, 6H, 3- and 3′-CH_3), 2.4–2.9 (m, 4 H, α-CH_2 of propionic ester and CH_2CH_2OH), 3.11 (t, J = 8 Hz, β-CH_2 of propionic ester), 3.4–4.0 (m, 3 H, CH_2OH and OH), 3.67 (s, OCH_3), 5.98 (s, methine H), 8.71 and 8.86 (each br s, NH); MS *m/e* (rel intensity) 512 (<1) M^+, 362 (<1) M^+ − 1-methylcamphene, 318 (100), 362 − CO_2, 287 (95), 318 − OCH_3.

Anal. ($C_{29}H_{40}N_2O_6$) C, H, N.

α-Methylfenchyl (2*R*)- and (2*S*)-3,3′-Dimethyl-4-(2-hydroxyethyl)-4′-(2-methoxycarbonylethyl)-5(2*H*)-2,2′-dipyrrylmethanone-5′-carboxylate (11). The foregoing 5(1*H*)-pyrromethenone (800 mg) was hydrogenated on 10% palladized charcoal according to the method described previously[15] for the corresponding 5′-*tert*-butyl ester, yielding 750 mg (93%) of *levorotatory* **11** as a mixture of epimers at C-2: IR (KBr) 3230, 2890, 1720, 1650, 1440, 1280 cm^{-1}; ^{1}H NMR (90 MHz) δ 0.94 (3 H), 1.07 (6 H) (each s, 1″- and 3″-CH_3), 1.4–2.1 (m, 7 H, fenchyl-CH_2 and 4″-H), 1.49 (s, 2″-CH_3), 1.96 and 2.00 (each s, 3- and 3′-CH_3), 2.3–2.9 (m, 4 H, α-CH_2 of propionic ester and CH_2CH_2OH), 2.9–3.3 (m, 4 H, β-CH_2 of propionic ester and CH_2 bridge), 3.6–3.9 (m, 3 H, CH_2OH and OH), 3.64 (s, OCH_3), 4.11 (m, 2-H), 6.91 and 7.08 (each br s, corresponding to two diastereomeric lactam NH), 9.33 (br s, pyrrole NH); MS *m/e* 514 (M^+), 374 (azafulvenium ion α-methylfenchyl ester[35]), 364 (M^+ − 1-methylcamphene), 320 (364 − CO_2), 224 (azafulvenium ion[36]), 180 (224 − CO_2).

α-Methylfenchyl (2*R*)- and (2*S*)-3,3′-Dimethyl-4-[2-(methylsulfonyloxy)ethyl]-4′-(2-methoxycarbonylethyl)-5(2*H*)-2,2′-dipyrrylmethanone-5′-carboxylate (12). The mixture of the foregoing 5(2*H*)-dipyrrylmethanones (750 mg) was treated with methanesulfonic acid chloride (0.4 mL) according to the method described previously[15] for the corresponding 5′-*tert*-butyl ester. Preparative TLC of the reaction product on silica gel using methylene chloride/methanol (96:4) as eluent afforded 740 mg (86%) of *levorotatory* **12** as a

mixture of epimers at C-2: ^{1}H NMR (90 MHz) δ 0.98 (3 H) and 1.10 (6 H) (each s, 1″- and 3″-CH_3), 1.4–2.1 (m, 7 H, fenchyl-CH_2 and 4″-H), 1.51 (s, 2″-CH_3). 1.98 and 2.00 (each s, 3- and 3′-CH_3), 2.2–3.6 (m, 8 H, α- and β-CH_2 of propionic ester, $CH_2CH_2OSO_2CH_3$ and CH_2 bridge), 2.97 (s, CH_3SO_2–), 3.68 (s, OCH_3), 4.20 (m, 2-H), 4.37 (t, J = 7 Hz, CH_2OSO_2–), 6.91 and 7.10 (each br s, NH corresponding to two diastereomeric lactams), 9.35 (br s, pyrrole NH); MS *m/e* 592 (M^+), 496 ($M^+ - CH_3SO_3H$), 346 (496 − 1-methylcamphene), 302 (346 − CO_2).

(+)- and (−)-α-Methylfenchyl 3,3′-Dimethyl-4-[2-(*p*-chlorophenylselenyl)ethyl]-4′-(2-methoxycarbonylethyl)-5(2*H*)-2,2′-dipyrrylmethanone-5′-carboxylate (13a and 13b, Respectively). To the yellow solution of bis(4-chlorophenyl) diselenide[19] (200 mg) in ethanol (80 mL), sodium borohydride (40 mg) was slowly added. After decolorization was complete, the solvent was removed under reduced pressure and the residue was treated with a solution made from the above mixture of mesylates (240 mg) in tetrahydrofuran (80 mL). The mixture was stirred for 20 min at room temperature and then worked up as described in ref 15 for the corresponding 5′-*tert*-butyl ester, yielding 30 mg of starting material and 148 mg (52%) of **13** as a mixture of epimers at C-2. By fractional crystallization of the latter from ether, 70 mg of dextrorotatory **13a** was obtained: mp 139–140 °C; $[\alpha]^{25}_D$ +56.8° (0.74 g/L in methanol); ORD (80 mg/L in methanol) λ [M] 600 (+1280), 350 (+3900), 290 (+14 450), 277 (0), 265 (−3400), 260 (0), 230 nm (+13 600°); IR (KBr) 3130, 2830, 1710, 1640, 1430, 1270 cm^{-1}; ^{1}H NMR (90 MHz) δ 0.97 (3 H) and 1.07 (6 H) (each s, 1″- and 3″-CH_3), 1.3–2.1 (m, 7 H, fenchyl-CH_2 and 4″-H), 1.49 (s, 2″-CH_3), 1.89 and 1.96 (each s, 3- and 3′-CH_3), 2.2–2.7 (m, 4 H, α-CH_2 of propionic ester and CH_2CH_2Se), 2.8–3.2 (m, 6 H, β-CH_2 of propionic ester, CH_2Se and CH_2 bridge), 3.65 (s, OCH_3), 4.00 (m, 2-H), 6.41 (br s, lactam NH), 7.39 (m, 4 H, phenyl H), 9.01 (br s, pyrrole NH); MS *m/e* 688 (M^+), 538 (M^+ − 1-methylcamphene), 494 (538 − CO_2), 224 (azafulvenium ion[36]), 180 (224 − CO_2) (base peak).

Anal. ($C_{35}H_{45}ClN_2O_5Se$) C, H, N.

The mother liquors contained mainly the levorotatory selenide **13b** which was obtained as an oil, $[\alpha]^{25}_D$ −75.4° (0.35 g/L in methanol).

(+)-α-Methylfenchyl 3,3′-Dimethyl-4′-(2-methoxycarbonylethyl)-4-vinyl-5(2*H*)-2,2′-dipyrrylmethanone-5′-carboxylate (14). Pure **13a** (70 mg) was reacted for 9 h with 30% hydrogen peroxide (0.25 mL) according to the procedure described previously[15] for the corresponding 5′-*tert*-butyl ester, yielding 40 mg (80%) of crystalline **14**: mp 180–182 °C; $[\alpha]^{25}_D$ +193.4° (79 mg/L in methanol); ORD (79 mg/L in methanol) λ [M] 600 (+950), 400 (+1640), 297 (+15 780), 282 (0), 270 (−15 140), 245 (0), 235 mm (+2520°); IR (KBr) 3330, 2880, 1730, 1650, 1440, 1420, 1290 cm^{-1}; ^{1}H NMR (90 MHz) δ 1.00 (3 H) and 1.09 (6 H) (each s, 1″- and 3″-CH_3), 1.3–2.1 (m, 7 H, fenchyl-CH_2 and 4″-H), 1.51 (s, 2″-CH_3), 2.00 and 2.07 (each s, 3- and 3′-CH_3), 2.3–3.2 (m, 6 H, α- and β-CH_2 of propionic ester and CH_2 bridge), 3.67 (s, OCH_3), 4.09 (m, 2-H), 5.41 (dd, J_{AX} = 12, J_{BX} = 2 Hz, H_X[37]), 6.24 (dd, J_{AB} = 18, J_{BX} = 2 Hz, H_B[37]), 6.42 (dd, J_{AB} = 18, J_{AX} = 12 Hz, H_A[37]), 6.62 (br s, lactam NH), 9.24 (br s, pyrrole NH); MS *m/e* (rel intensity) 496 (2) M^+, 374 (14) azafulvenium ion α-methylfenchyl ester,[35] 346 (7) M^+ − 1-methylcamphene, 302 (6), 346 − CO_2, 224 (100) azafulvenium ion,[36] 180 (29), 224 − CO_2.

(+)-(2*R*)-3,3′-Dimethyl-5′-formyl-4′-(2-methoxycarbonylethyl)-4-vinyl-5(2*H*)-2,2′-dipyrrylmethanone (19). To a solution of the foregoing α-fenchyl ester (60 mg) in trifluoroacetic acid (3 mL) was added trimethyl orthoformate (0.3 mL) and the mixture was stirred for 40 min at room temperature. Thereupon, the solution was diluted with methylene chloride (50 mL) and shaken with saturated aqueous $NaHCO_3$. The organic phase was filtered through methylene chloride soaked filter paper and the solvent was removed on a rotary evaporator to yield 33 mg (83%) of crystalline **19.** Recrystallization from methylene chloride/*n*-hexane provided analytically pure aldehyde **19:** mp 138–139 °C; $[\alpha]^{25}_D$ +291.4° (73 mg/L in methanol); ORD (73 mg/L in methanol) λ [M] 600 (+1220), 400 (+2790), 325 (+11 260), 306 (0), 280 (−10 350), 270 (−11 260), 257 (0), 240 nm (+8100); IR (KBr) 3200, 2880, 1730, 1680, 1630, 1450, 1170 cm^{-1}; ^{1}H NMR (90 MHz) δ 1.98 (s, 3′-CH_3), 2.09 (s, 3-CH_3), 2.55 (t, J = 7 Hz, α-CH_2 of propionic ester), 2.7–3.3 (m, 4 H, β-CH_2 of propionic ester and CH_2 bridge), 3.64 (s, OCH_3), 4.22 (m, 2-H), 5.38 (dd, J_{AX} = 11, J_{BX} = 3 Hz, H_X[37]), 6.21 (dd, J_{AB} = 17, J_{BX} = 3 Hz, H_B[37]), 6.41 (dd, J_{AB} = 17, J_{AX} = 11 Hz, H_A[37]), 7.30 (br s, lactam NH), 9.40 (s, formyl H), 11.00 (br s, pyrrole NH); MS *m/e* (rel intensity) 330 (100) M^+, 301 (17) M^+ − CHO, 299 (14) M^+ − OCH_3, 208 (26) 5-formyl-3-methyl-2-methylene-2*H*-pyrrolium-4-propionic acid methyl ester.

(+)-(2*R*,16*R*)- and (+)-(2*S*,16*R*)-3-Ethylidene-2,7,13,17-tetramethyl-18-vinyl-1,19-dioxo-1,2,3,15,16,19,21,24-octahydro-22*H*-bilin-8,12-dipropionic Acid Dimethyl Ester [(+)-(2*R*,16*R*)- and (+)-(2*S*,16*R*)-Phycoerythrobilin Dimethyl Ester] (21a and 21b, Respectively). The foregoing dipyrrylmethanone aldehyde (40 mg) and racemic **20**[14] (54 mg) were dissolved together in trifluoroacetic acid (4 mL) and the mixture was stirred for 30 min at room temperature. After methanol (4 mL) was added, stirring was continued for a further 40 min at room temperature. Thereupon, methylene chloride (50 mL) was added and the solution was shaken with saturated aqueous $NaHCO_3$. The organic layer was dried by filtration through methylene chloride soaked filter paper and the solvent was evaporated under reduced pressure. Preparative TLC, of the residue on silica gel, using CCl_4/ethyl acetate (1:1) as eluent, afforded two epimeric phycoerythrobilin dimethyl esters. Elution of the band at R_f 0.22 with methanol gave 20 mg (27%) of (+)-(2*R*,16*R*)-phycoerythrobilin dimethyl ester (**21a**). Recrystallization from acetone gave pure **21a**, mp 182–184 °C (lit.[7] 183–184 °C from acetone/water), whose analytical data, including R_f value, mixture melting point, as well as UV/vis, IR, ^{1}H NMR, CD, and ORD spectra (cf. Figure 1) were identical with those of an authentic sample obtained from denatured *C*-phycoerythrin by treatment with boiling methanol: CD (52 mg/L in CH_2Cl_2[38]) λ (Θ) 600 (+74 300), 440 (+1700), 390 (+11 600), 360 (0), 322 (−66 [])(= 262 (−8300), 250 nm (−26 400); ORD (17 mg/L in CH_2Cl_2[38]) λ [M] 650 (+35 800), 620 (+65 800), 600 (0), 540 nm (−52 700°); ORD (52 mg/L in CH_2Cl_2[38]) λ [M] 415 (−31 000), 350 (−66 400), 280 (0), 260 nm (−18 800°); UV/vis λ_{max} (log ε) 305 (sh), 318 (4.28), 331 (sh), 500 (sh), 530 nm (4.53); UV/vis [5% (w/v) methanolic HCl] λ_{max} (log ε) 328 (4.27), 386 (sh), 500 (sh), 594 nm (4.69); IR (CCl_4) 3440, 3230, 2950, 2910, 1730, 1695, 1600, 1430 cm^{-1}; ^{1}H NMR (100 MHz) δ ($CDCl_3$ + 1 drop CD_3OD) 1.43 (d, J = 7 Hz, 2-CH_3), 1.91 (dd, ^3J = 7.6, ^5J = 1.2 Hz, ethylidene CH_3), 1.98 (3 H) and 2.03 (6 H) (each s, CH_3), 2.4–2.7 (m, 4 H, α-CH_2 of propionic esters), 2.8–3.0 (m, 6 H, β-CH_2 of propionic esters and 15-CH_2), 3.24 (q, J = 7 Hz, 2-H), 3.64 and 3.66 (each s, OCH_3), 4.28 (m, 16-H), 5.36 (dd, J_{AX} = 12, J_{BX} = 2 Hz, H_X[37]), 5.84 (s, 5-H), 6.17 (dd, J_{AB} = 18, J_{BX} = 2 Hz, H_B[37]), 6.40 (dq, ^3J = 7.6, ^4J = 2.2 Hz, ethylidene H), 6.40 (dd, J_{AB} = 18, J_{AX} = 12 Hz, H_A[37]), 6.68 (s, 10-H); MS *m/e* (rel intensity) 614 (51) M^+, 599 (8) M^+ − CH_3, 492 (100) M^+ − 4-methyl-2-oxo-3-vinyl-3-pyrrolinyl radical, 478 (12) M^+ − (4-methyl-2-oxo-3-vinyl-3-pyrrolin-3-yl)methyl radical, 302 (78), 180 (100) azafulvenium ion[36] − CO_2.

Elution of the band at R_f 0.18 with methanol gave 20 mg (27%) of (+)-(2*S*,16*R*)-phycoerythrobilin dimethyl ester, which crystallized from methylene chloride/*n*-hexane: mp 140–142 °C; CD (51 mg/L in CH_2Cl_2[38]) λ (Θ) 600 (+76 700), 440 (+2500), 390 (+10 100), 360 (0), 322 (−50 600), 272 (−4200), 260 nm (−16 900°); ORD (16 mg/L in CH_2Cl_2[38]) λ [M] 650 (+34 200), 620 (+55 400), 600 (0), 540 nm (−54 500°); ORD (85 mg/L in CH_2Cl_2[38]) λ [M] 415 (−26 200), 340 (−55 400), 295 (−1500), 270 nm (−16 900°); UV/vis λ_{max} (log ε) 305 (sh), 318 (4.30), 331 (sh), 500 (sh), 530 (4.54); UV/vis [5% (w/v) methanolic HCl] λ_{max} (log ε) 328 (4.27), 386 (sh), 500 (sh), 594 (4.70); IR (CCl_4) 3440, 3230, 2950, 2910, 1730, 1690, 1600, 1430 cm^{-1}; ^{1}H NMR (100 MHz) δ ($CDCl_3$ + 1 drop CD_3OD) 1.42 (d, J = 7 Hz, 2-CH_3), 1.90 (dd, ^3J = 7.6, ^5J = 1.2 Hz, ethylidene CH_3), 2.00 (3 H) and 2.04 (6 H) (each s, CH_3), 2.4–2.7 (m, 4 H, α-CH_2 of propionic esters), 2.8–3.0 (m, 6 H, β-CH_2 of propionic esters and 15-CH_2), 3.22 (q, J = 7 Hz, 2-H), 3.66 and 3.68 (each s, OCH_3), 4.28 (m, 16-H), 5.36 (dd, J_{AX} = 12, J_{BX} = 2 Hz, H_X[37]), 5.84 (s, 5-H), 6.15 (dd, J_{AB} = 18, J_{BX} = 2 Hz, H_B[37]), 6.40 (dq, ^3J = 7.6, ^4J = 2.2 Hz, ethylidene H), 6.42 (dd, J_{AB} = 18, J_{AX} = 12 Hz, H_A[37]), 6.68 (s, 10-H); MS *m/e* (rel intensity) 614 (18) M^+, 492 (43) M^+ − 4-methyl-2-oxo-3-vinyl-3-pyrrolinyl radical, 478 (8) M^+ − (4-methyl-2-oxo-3-vinyl-3-pyrrolin-3-yl)methyl radical, 302 (50), 180 (100) azafulvenium ion[36] − CO_2.

(+)-(4*R*,16*R*)-3-Ethyl-2,7,13,17-tetramethyl-18-vinyl-1,19-dioxo-1,4,5,15,16,19,21,24-octahydro-22*H*-bilin-8,12-dipropionic Acid Dimethyl Ester Hydrochloride [(+)-(4*R*,16*R*)-[18-Vinyl]mesourobilin IXα Dimethyl Ester Hydrochloride] (23). Aldehyde **19** (8 mg) was condensed with **22**[17] (8 mg, $[\alpha]_{578}^{25}$ +40° in methanol) in trifluoroacetic acid according to the previously described method,[15] yielding 7 mg (44%) of hydrochloride **23** whose analytical data, including R_f value (0.34 on silica gel, benzene/*n*-hexane/methanol, 50:10:6); CD, ORD, UV/vis, IR, and ^{1}H NMR spectra were identical with those

of a sample of (+)-(4*R*,16*R*)-[18-vinyl]mesourobilin IXα dimethyl ester synthesized earlier by us by an independent route.[15]

(−)-2(*E*)-Ethylidene-3(*R*)-methylsuccinimide (24). To a solution of $NaHSO_4$ (250 mg) and sodium dichromate (250 mg) in water (6 mL) was added a solution of **21a** (9 mg) in tetrahydrofuran (2 mL) and the mixture was stirred for 2 h at room temperature. After dilution with water (10 mL), the mixture was extracted with methylene chloride (7 × 10 mL) and the combined organic phases were dried by filtration through methylene chloride soaked filter paper. After evaporation of the solvent, under reduced pressure, preparative TLC of the residue on silica gel, using carbon tetrachloride/ethyl acetate/cyclohexane (5:3:1) as eluent, yielded 1 mg (56%) of **24:** CD (83 mg/L in methanol) λ (Θ) 260 (−1500), 250 (−5900), 242 (−11 700), 235 (−7600), 230 nm (0°).

(+)-2(*E*)-Ethylidene-3(*S*)-methylsuccinimide (25) was obtained as above by oxidative degradation of **21b** (6 mg) in 34% yield: CD (230 mg/L in methanol) λ (Θ) 260 (+1100), 250 (+5800), 242 (+12 000), 235 (+7400), 230 nm (0°).

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References and Notes

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(37) The protons of the vinyl group have been assigned as given on formulas **14–19.**
(38) Before use, the solvent was degassed and shaken with 1 N aqueous HCl.