Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 21, pp. 2517-2522, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00367-X

LONG-WAVELENGTH ABSORBING DERIVATIVES OF PHYCOCYANOBILIN: NEW STRUCTURAL ASPECTS ON PHYTOCHROME

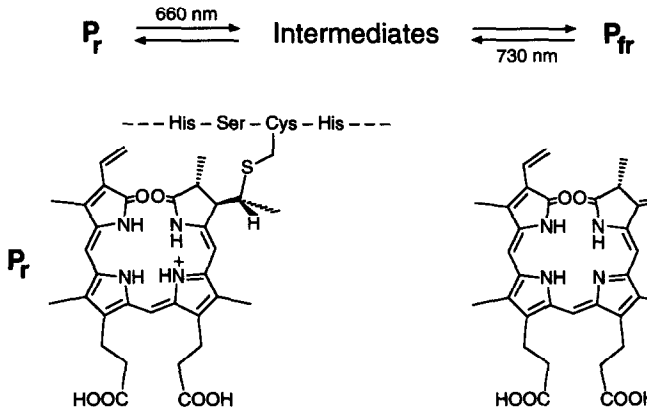
Ronald Micura and Karl Grubmayr*

Institut für Chemie, Johannes Kepler Universität Linz, Altenbergerstraße 69, A-4040 Linz, Austria

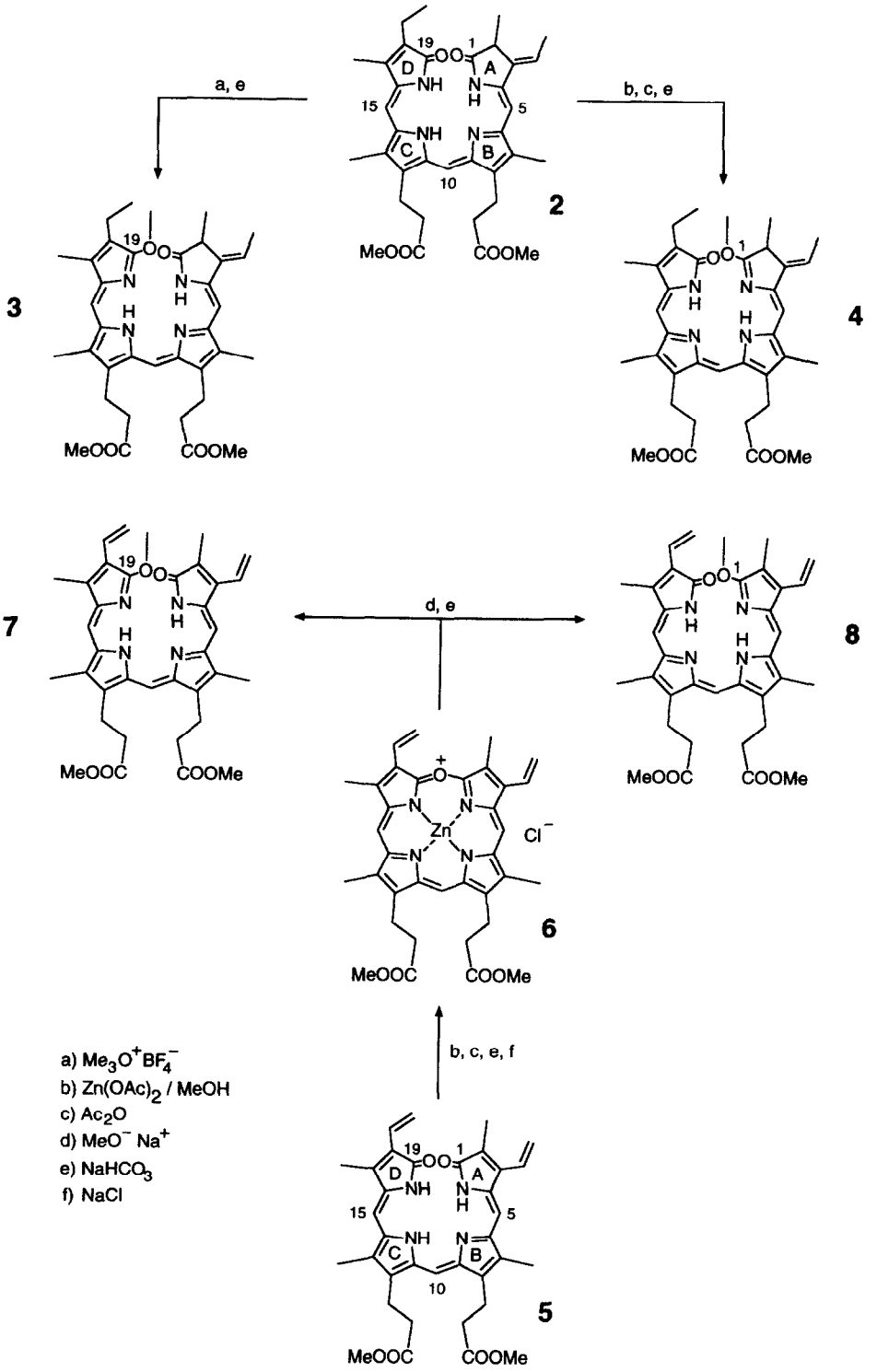
Abstract. Site selective conversion of phycocyanobilin dimethyl ester **2** to the ring-A methylimino ester **4** is accompanied by a bathochromic shift of the long wavelength absorption of more than 100 nm. Compared to its ring-D counterpart **3** and the biliverdin imino esters **7** and **8**, **4** might yield new insights into phytochrome activity.

Morphogenetic processes of higher plants are triggered by the photochemically initiated conversion of the red light absorbing biliprotein phytochrome P_r to the far-red absorbing form P_{fr}[1]. Conversion presumably starts with Z/E photoisomerization[2] of its bilindione chromophore, phytochromobilin **1**. A series of dark reactions[3] follows resulting in a bathochromic shift of the long wavelength absorption from 660 nm to 730 nm. Detailed structural assignments of the dark reaction intermediates and P_{fr} itself are not accessible so far[4]. Therefore our efforts concentrate on chemical transformations of bilindione chromophores that are in accordance with these shift characteristics.

Starting from the readily available dimethyl esters of phycocyanobilin **2** and biliverdin **5** our investigations are based on a comparison of their imino esters. In this letter we present phycocyanobilin ring-A methylimino ester **4** as a model compound for phytochrome activity with regard to potential chromophore protein interactions.



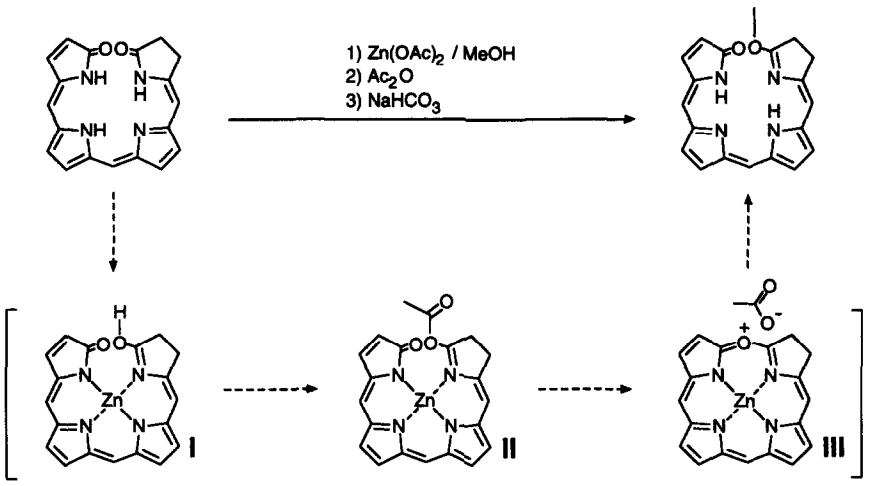
Preparation of biliverdin monomethylimino esters had been accomplished using Fuhrhop's protocol[5]. During this two step synthesis oxoniaporphinato zinc complex **6** was isolated as a stable compound that could be converted by methoxide to a mixture of the regioisomeres **7** and **8** in high yield. We succeeded in the separation of **7**[6] and **8**[7] by MPLC (silica; dichloromethane : ethyl acetate = 15 : 1; **7** : **8** = 1,5 : 1; R_f: **7** < **8**) enabling structural assignment subsequently.


a, e
b, c, e
d, e
b, c, e, f
2
3
4
5
6
7
8
MeOOC
COOMe
Cl⁻
a) $Me_3O^+BF_4^-$
b) $Zn(OAc)_2$ / MeOH
c) Ac_2O
d) MeO^-Na^+
e) $NaHCO_3$
f) NaCl

Preparation of phycocyanobilin monomethylimino esters is different. Ring-A imino ester **4** is formed selectively in a one pot reaction. Thereby acetic anhydride (0.8 ml; 8.4 mmol) was added to a solution of zinc acetate (25 mg; 114 mmol) in methanol (1 ml) and **2** (50 mg; 81 mmol) in chloroform (4 ml). After reflux (15 min) and workup with aqueous sodium hydrogen carbonate **4**[8] was purified by column chromatography (silica; dichloromethane : ethyl acetate = 7 : 1; R_f: **4** > **2**; 11.2 mg **4** (22%), 25 mg **2** (50%)).

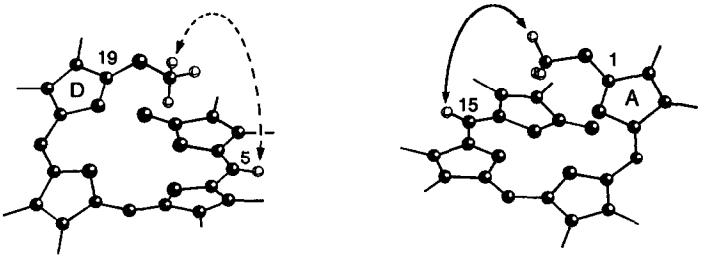
Synthesis of Ring-D imino ester **3** could not be achieved along these lines. However, **2** (50 mg; 81 mmol) was converted to **3** selectively by use of trimethyloxonium tetrafluoroborate (38 mg; 238 mmol) in dichloromethane (3 ml). After stirring (2h; room temperature) and workup with aqueous sodium hydrogen carbonate **3**[9] was purified by column chromatography (silica; dichloromethane : ethyl acetate = 6 : 1; R_f: **3** > **2**; 10.5 mg **3** (20%), 39 mg **2** (80%)).

Differences of imino ester preparations are based on the different oxidation states of bilindione **5** and 2,3-dihydrobilindione **2**. Concerning the mechanism of the acetic anhydride procedure oxonia chlorinato zinc complexes **III** have to be taken into account beside the well characterized and stable oxoniaporphinato

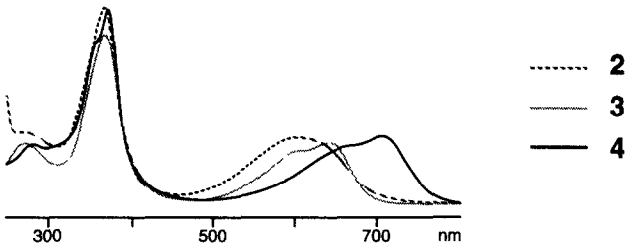


analogues[5] like **6**. Oxonia chlorinato zinc complexes could not be isolated. They seem to be of higher reactivity. Consequently, the use of methanol instead of methoxide is sufficient for ring opening in a regioselective way[10] yielding **4** exclusively. Furthermore, regioselective formation of ring-D imino ester **3** with trimethyloxonium tetrafluoroborate is derived from the higher nucleophilicity of the ring-D lactam carbonyl group[11].

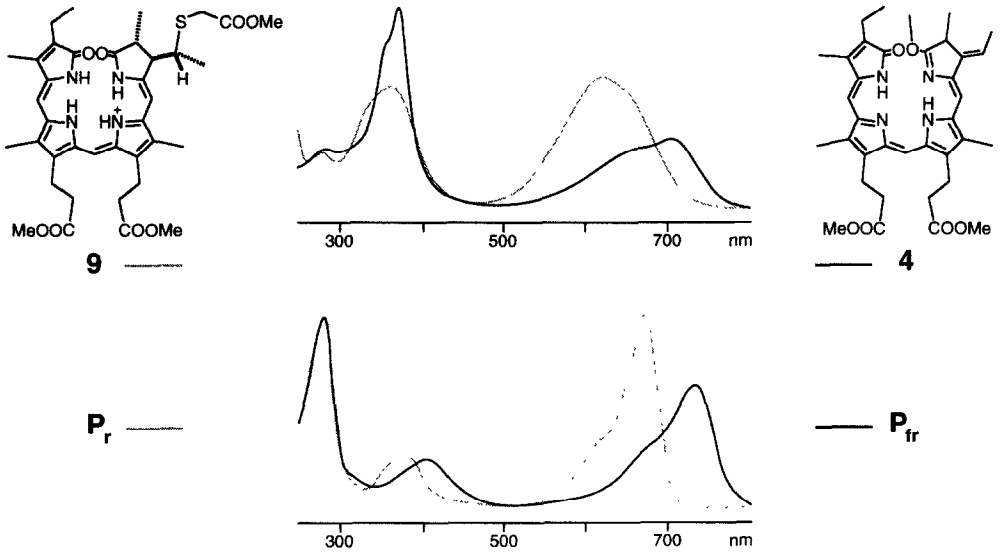
Assignment of the ring-A imino esters **4** and **8** results from the observation of a distinct NOESY cross peak (CH_3O-C1↔H-C15). The corresponding cross peak (CH_3O-C19↔H-C5) for ring-D imino ester assignment of **3** and **7** is observable as well but of lower intensity. Thus, *syn* conformation of all the imino esters is established together with the helical geometry of the chromophore. Further evidence for such a chromophore



arrangement arises from the ^{1}H-NMR chemical shift values of the NH signals around 12.5 and 10.0 ppm, indicating hydrogen bridges within the A-B and C-D moieties. These findings are in good accordance with the crystal structure of the monomethylimino ester of aetiobiliverdin IV-γ[12].



Despite the geometrical similarities of all these imino esters, significant differences of their absorption spectra can be observed. In particular, spectroscopic properties of phycocyanobilin ring-A imino ester **4** are quite outstanding: long range absorption maximum at 705 nm reveals that a bathochromic shift of more than 100 nm can be accomplished by a simple chemical transformation of phycocyanobilin **2**. In comparison with ring-D imino ester **3**, the extent of the bathochromic shift is less than half that value. With respect to biliverdin imino esters **7** and **8**, moves of the absorption maxima are even smaller but of comparable values (**5** → **7**: 25 nm; **5** → **8**: 29 nm). Consequently, the large bathochromic shift is a characteristic feature only for imino ester transformation at the ring-A site of 2,3-dihydro-chromophores. These findings correlate well with simply substituted monomethylimino esters of 2,3-dihydrobilindiones prepared by multi step synthesis[13].



Absorption behavior and structure of **4** encourage to think about parallels to the $P_r \rightarrow P_{fr}$ pathway, which is characterized spectroscopically by the move of the absorption maximum from 660 nm to 730 nm. An analogous shift tendency can be found comparing UV-Vis spectra of protonated phycocyanobilin thiol adduct **9**[14] and imino ester **4**. Correlating their structures to the chromophores of P_r and P_{fr}, a hypothesis for phytochrome activity can be proposed by special chromophore protein interactions: detachment of the cystein^{321}-thiol by elimination and attachment of a hydroxylic side-chain by imino ester formation. In principle, reversibility is

possible by thiol addition and imino ester hydrolysis. Latter is in good accordance with the acid catalyzed hydrolysis of **4** (chloroform / water - trifluoroacetic acid; 20 °C; 30 sec; 100%). Nevertheless, other nucleophilic side-chains might act in an analogous way to the hydroxy group and hence could form a covalent chromophore protein linkage involving C1 as well. With respect to the absorption maxima our model system based on phycocyanobilin lacks the additional conjugation of the ring-D vinyl group. Therefore, the 25 nm gap up to 730 nm may be at least diminished by the use of phytochromobilin derivatives.

In particular, our studies concentrate on chemical transformations of the ring-A moiety. They are very close to the proposals for phytochrome phototransformation made by Lagarias and Rapoport[15]. However, the concept is open to many features of commonly accepted theories on phytochrome activity[1,2,16] including dark reaction intermediates[3] as well. At the moment our investigations are extended to amino acid derivatives of **2** to check the proposed conception in a more biomimetic way.

Acknowledgment:
This work was supported by the *Fonds zur Förderung der wissenschaftlichen Forschung* in Austria (FWF-Project No. P9166-CHE).

References and Notes:

1. (a) Sage, L. C. *Pigment of the Imagination. A History of Phytochrome Research*; Academic Press, Inc.: San Diegeo, 1992.
(b) Rüdiger, W.; Thümmler, F. *Angew. Chem.* **1991**, *103*, 1242.
(c) Schaffner, K.; Braslavsky, S. E.; Holzwarth, A. R. Protein Environment, Photophysics and Photochemistry of Prosthetic Biliprotein Chromophores. In *Frontiers in Supramolecular Organic Chemistry and Photochemistry;* Schneider, H.-J.; Dürr, H. Eds.; VCH-Verlagsgesellschaft: Weinheim, Germany 1991; pp. 421-452.
2. Rüdiger, W.; Thümmler, F.; Cmiel, E.; Schneider, S. *Proc. Natl. Acad. Sci. USA* **1983**, *80*, 6244.
3. (a) Inoue, Y.; Rüdiger, W.; Grimm, R.; Furuya M. *Photochem. Photobiol.* **1990**, *52*, 1077.
(b) Scurlock, R. D.; Braslavsky, S. E.; Schaffner, K. *Photochem. Photobiol.* **1993**, *57*, 690.
(c) Zhang, C.-F.; Farrens, D. L.; Björling, S. C.; Song, P.-S.; Kliger, D. S. *J. Amer. Chem. Soc.* **1992**, *114*, 4569.
(d) Song, P.-S.; Singh, P. R.; Tamai, N.; Yamazaki, I.; Yamazaki, S.; Tokutomi, S.; Furuya, M. *Biochemistry* **1989**, *28*, 3265.
(e) Eilfeld, P. H.; Vogel, J.; Maurer, R.; Eilfeld, P. G. *J. Photochem. Photobiol. B* **1989**, *3*, 209.
4. Rüdiger, W. *Photochem. Photobiol.* **1992**, *56*, 803.
5. (a) Fuhrhop, J.-H.; Krüger, P; Sheldrick, W. S. *Liebigs Ann. Chem.* **1977**, *1977*, 339.
(b) Fuhrhop, J.-H.; Krüger, P. *Liebigs Ann. Chem.* **1977**, *1977*, 360.
6. **7**: ^{1}H-NMR (360 MHz; $CDCl_3$; J (Hz)): δ = 12.82 (s, 1H, NH); 10.31 (s, 1H, NH); 6.74 (s, 1H, H-(C10)); 6.60 (dd, 1H, J_Z = 11.8, J_E = 17.9, H-(C3')); 6.45 (dd, 1H, J_Z = 11.5, J_E = 17.6, H-(C18')); 6.41 (s, 1H, H-(C15); 5.93 (s, 1H, H-(C5)); 5.66 (dd - overlapped, 1H, J = 1.5, J_E = 17.9, H-(C3')); 5.63 (dd - overlapped, 1H, J = 1.5, J_Z = 11.8, H-(C3")); 5.61 (dd - overlapped, 1H, J = 2.1, J_E = 17.6, H-(C18")); 5.23 (dd, 1H, J = 2.1, J_Z = 11.5, H-(C18"); 3.94 (s, 3H, CH_3O-(C19)); 3.68, 3.66 (2s, 6H, CH_3O-(C8'"), CH_3O-(C12'")); 2.93, 2.89 (2 x triplet like, 4H, CH_2-(C8), CH_2-(C12)); 2.53, 2.52 (2 x triplet like, 4H, CH_2-(C8')+ CH_2-(C12')); 2.17 (s, 3H, CH_3-(C17)); 2.14 (s, 3H, CH_3-(C13)); 2.05 (s, 3H, CH_3-(C7)); 1.93 (s, 3H, CH_3-(C2)).
^{13}C-NMR (90 MHz; $CDCl_3$): δ = 176.62; 173.13 (2x); 170.66; 165.82; 150.83; 149.43; 143.04; 142.03; 141.91; 138.98; 137.05; 132.96; 132.61; 132.14; 130.28; 126.77; 126.41 (C3'); 126.35 (C18'); 125.56; 122.03 (C3"); 116.39 (C18"); 115.58 (C10); 109.40 (C15); 97.62 (C5); 55.89 ($\underline{C}H_3O$-C19); 51.64 ($\underline{C}H_3O$-C8'") + $\underline{C}H_3O$-C12'"); 35.31 (C8"+ C12"); 19.96, 19.71 (C8', C12'); 9.87 (C17'); 9.70 (C7'); 9.60 (C2'); 8.93 (C13').
UV-Vis ($CHCl_3$): nm (ε) = 686 (14100); 656 (sh, 12600); 384 (40900); 330 (sh, 21900); 281 (18000).
7. **8**: ^{1}H-NMR (360 MHz; $CDCl_3$; J (Hz)): δ = 12.94 (s, 1H, H-(N22)); 10.16 (s, 1H, H-(N24)); 6.72 (s, 1H, H-(C10)); 6.65 (dd, 1H, J_Z = 11.5, J_E = 17.8, H-(C3')); 6.52 (dd, 1H, J_Z = 11.5, J_E = 17.6, H-(C18')); 6.46 (s, 1H, H-(C5); 6.19 (dd, 1H, J = 1.8, J_E = 17.6, H-(C18")); 5.82 (s, 1H, H-(C15)); 5.53

(dd, 1H, J = 1.8, J_E = 17.8, H-(C3")); 5.45 (dd, 2H, J = 1.8, J_Z = 11.5, H-(C3") + H-(C18")); 3.96 (s, 3H, CH_3O-(C1)); 3.68, 3.67 (2s, 6H, CH_3O-(C8'''), CH_3O-(C12''')); 2.91, 2.87 (2 x triplet like, 4H, CH_2-(C8), CH_2-(C12)); 2.54, 2.53 (2 x triplet like, 4H, CH_2-(C8') + CH_2-(C12')); 2.14 (s, 3H, CH_3-(C17)); 2.12 (s, 3H, CH_3-(C7)); 2.05 (s, 3H, CH_3-(C13)); 1.83 (s, 3H, CH_3-(C2));
^{13}C-NMR (90 MHz; $CDCl_3$): δ = 177.37; 173.13 (2x); 169.56; 165.52; 150.86; 148.97; 143.70; 142.04; 141.14; 139.16; 137.31; 132.78; 132.37; 132.25; 128.21; 127.61 (C3'); 126.93; 126.13; 125.94 (C18'); 125.41; 120.83 (C18"); 119.32 (C3"); 115.78 (C10); 109.50 (C5); 97.61 (C15); 55.99 ($\underline{C}H_3O$-C1); 51.62 ($\underline{C}H_3O$-C8''') + $\underline{C}H_3O$-C12'''); 35.33, 35.30 (C8", C12"); 19.96, 19.70 (C8', C12'); 9.70 (C13'); 9.31 (C17'); 9.25 (C2'); 8.89 (C7').
UV-Vis ($CHCl_3$): nm (ε) = 690 (14500); 656 (sh, 12700); 381 (45100); 317 (20700); 280 (17600).

8. **4**: ^{1}H-NMR (360 MHz; $CDCl_3$; J (Hz)): δ = 12.2 (broad, 1H, H-(N22)); 9.66 (s, 1H, H-(N24)); 6.77 (s, 1H, H-(C10)); 6.18 (dq, 1H, J = 2.1, J = 7.2, H-(C3')); 6.09 (s, 1H, H-(C5)); 5.87 (s, 1H, H-(C15); 3.83 (s, 3H, CH_3O-(C1)); 3.67, 3.66 (2s, 6H, CH_3O-(C8'''), CH_3O-(C12''')); 3.40 (dq, 1H, J = 2.1, J = 7.5, H-(C2)); 2.95, 2.91 (2 x triplet like, 4H, CH_2-(C8), CH_2-(C12)); 2.54 (triplet like, 4H, CH_2-(C8') + CH_2-(C12')); 2.32 (q, 2H, J = 7.6, CH_2-(C18)); 2.09, 2.07 (2s, 6H, 3H, CH_3-(C7), CH_3-(C13), CH_3-(C17)); 1.87 (d, 3H, J = 7.2, CH_3-(C3'); 1.27 (d, 3H, J = 7.5, CH_3-(C2)); 1.06 (t, 3H, J = 7.6, CH_3-(C18')).
^{13}C-NMR (90 MHz; $CDCl_3$): δ = 182.95; 173.35; 173.25; 171.25; 161.84; 151.27; 147.89; 142.35; 140.82; 140.30; 140.20; 138.91; 134.73; 133.79; 130.78; 129.65; 121.35; 118.03 (C3'); 115.45 (C10); 97.41 (C15); 95.23 (C5); 56.53 ($\underline{C}H_3O$-C1); 51.60 ($\underline{C}H_3O$-C8''') + $\underline{C}H_3O$-C12'''); 40.16 (C2); 35.61, 35.49 (C8", C12"); 20.13, 19.87 (C8', C12'); 16.91 (C18'); 15.04 (C3"); 14.93 (C2'); 13.25 (C18"); 9.70, 9.25, 8.87 (C7', C13', C17').
UV-Vis ($CHCl_3$): nm (ε) = 705 (15200); 665 (sh, 13200); 373 (41400); 364 (sh, 37200); 232 (13100).
IR ($CHCl_3$): cm^{-1} = 3339; 1732; 1692; 1597; 1571.
MS (33 eV; 340 °C): m/e (%) = 628 (M^+, 65), 613 (75), 316 (54), 302 (76).

9. **3**: ^{1}H-NMR (360 MHz; $CDCl_3$; J (Hz)): δ = 12.61 (s, 1H, NH); 11.1 (broad, 1H, NH); 6.61 (s, 1H, H-(C10)); 6.37 (s, 1H, H-(C15)); 6.34 (dq, 1H, J = 2.2, J = 7.2, H-(C3')); 5.74 (s, 1H, H-(C5); 3.87 (s, 3H, CH_3O-(C19)); 3.68, 3.66 (2s, 6H, CH_3O-(C8'''), CH_3O-(C12''')); 3.05 (dq, 1H, J = 2.2, J = 7.5, H-(C2)); 2.91, 2.87 (2 x triplet like, 4H, CH_2-(C8), CH_2-(C12)); 2.54, 2.52 (2 x triplet like, 4H, CH_2-(C8') + CH_2-(C12')); 2.23 (q, 2H, J = 7.6, CH_2-(C18)); 2.13 (s, 3H, CH_3-(C13)); 2.08 (s, 3H, CH_3-(C17)); 2.03 (s, 3H, CH_3-(C7)); 1.89 (d, 3H, J = 7.2, CH_3-(C3');1.32 (d, 3H, J = 7.5, CH_3-(C2)); 1.02 (t, 3H, J = 7.6, CH_3-(C18')).
^{13}C-NMR (90 MHz; $CDCl_3$): δ = 177.10; 176.65; 173.32; 173.24; 166.99; 150.26; 149.43; 145.82; 141.66; 140.55; 136.34; 135.10; 132.50; 131.62; 131.01; 130.00; 124.40; 121.96 (C3'); 112.42 (C10); 108.28 (C15); 87.07 (C5); 55.52 ($\underline{C}H_3O$-C19); 51.62 ($\underline{C}H_3O$-C8''') + $\underline{C}H_3O$-C12'''); 38.01 (C2); 35.47, 35.37 (C8", C12"); 19.99, 19.77 (C8', C12'); 16.97 (C18'); 16.02 (C2'); 14.82 (C3"); 13.36 (C18"); 9.79 (C7'); 9.50 (C17'); 8.91 (C13').
UV-Vis ($CHCl_3$): nm (ε) = 642 (14300); 609 (sh, 12400); 369 (39100); 272 (14000).
IR ($CHCl_3$): cm^{-1} = 3305; 1732; 1619; 1590.
MS (33 eV; 340 °C): m/e (%) = 628 (M^+, 73), 613 (100).

10. Iturraspe, J.; Gossauer, A. *Photochem. Photobiol.* **1991**, *54*, 43.
11. Grubmayr, K.; Kapl, G. *Monatsh. Chem.* **1988**, *119*, 605.
12. Kratky, C.; Jorde, C.; Falk, H.; Thirring, K. *Tetrahedron* **1983**, *39*, 1859.
13. Falk, H; Zrunek, U. *Monatsh. Chem.* **1983**, *114*, 983.
14. Stumpe, H.; Müller, N.; Grubmayr, K. *Tetrahedron Lett.* **1993**, *34*, 4165.
15. Lagarias, J. C.; Rapoport, H. *J. Amer. Chem. Soc.* **1980**, *102*, 4821.
16. (a) Singh, B. R.; Song, P.-S. *Photochem. Photobiol.* **1990**, *52*, 249.
(b) Weller, J.-P.; Gossauer, A. *Chem. Ber.* **1980**, *113*, 1603.
(c) Sommer, D.; Song, P.-S. *Biochemistry* **1990**, *29*, 1943.

(*Received in Belgium* 28 *June* 1994; *accepted* 29 *August* 1994)