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Substitution of the Flavin Chromophore with Lipophilic Side Chains: A Novel Membrane Redox Label

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Summary. The synthesis of "amphiphilic" flavins substituted with C_{18}-hydrocarbon sidechains in positions 3, 5, 7, 8 and 10 is described. 3-, 7-, and 10-amphiflavins were obtained by new total syntheses. Furthermore, 3-amphiflavin was obtained by C_{18}-alkylation of natural flavin in the oxidized state, whereas 5-amphi(dihydro)flavin was obtained by alkylation under reducing conditions.

In the course of these studies, a novel, selective oxidation reaction was found taking place with the 8-methyl group of natural flavins. In this way lumiflavin and riboflavin derivatives could be converted directly to flavin-8-nor-8-carboxylic acids or the corresponding alkyl esters.

The new flavin derivatives lend themselves for incorporation into lipid vesicles, thus yielding the basis for model studies of "anisotropic flavin chemistry" and redox transfer through membranes, as described in the concomitant paper (Schmidt, W., Hemmerich, P. 1981). *J. Membrane Biol.* **59**:129. The new flavins are characterized by means of absorption, fluorescence, and proton nuclear magnetic resonance spectroscopy.

Flavoproteins are an ubiquitous class of redox enzymes, performing a wealth of reactions which appear to be disorderly at first sight. The same type of cofactor mediates biological activities as different as CH- and SH-dehydrogenation [31], single electron transfer [11], dioxygen activation [18], and transformation of light signals (phototropism, bluelight reception) [17]. Among these activities only two, namely, single electron transfer and, curiously enough, dioxygen activation, are encountered with the free flavin coenzyme in homogeneous solution. Massey and Hemmerich [13] have recently shown that the environment, i.e., in biological systems the apoprotein, regulates the type of flavin reactivity by regiospecific hydrogen bridges towards either the N(1)/O(2α)- or the N(5)-site of the flavin nucleus. It appears, furthermore, that hydrophobic shielding of parts of the flavin moiety has a great influence upon the reactivity [29]. Hence, we have to face the fact that, under biological conditions, the flavin coenzyme is located in an anisotropic environment. In order to create model systems for this situation, we have set out to synthesize so-called amphiflavins, which can be built into lipid vesicles by their hydrophobic sidechain and which will give rise to an anisotropic model environment for the flavin nucleus, depending on its site of hydrophobic substitution. "Amphiflavins" are defined by the highly polar heteroaromatic flavin nucleus, covalently connected to a highly unpolar long aliphatic sidechain. In the present paper we describe the synthesis of representative amphiflavins bearing their longchain substituents alternatively at each one of the, roughly, four sides or corners of the flavin chromophore. In the concomitant investigations on flavin-loaded vesicles [29, 30] we study the changes of physical and chemical flavin properties as induced by the anisotropic environment.

Materials and Methods

Solvents and reagents were commercial products and were purified and dried according to standard methods described in Organikum [25] or Riddick and Bunker [28]. Dichloromethane was purified according to Ratcliffe and Rodehorst [27]. Potassium carbonate was activated for 12 hr at 120 °C and 10^{-2} Torr over potassium hydroxide.

All melting points are uncorrected and were run on a Kofler Heating block. Elemental analyses were performed by Hoffmann La Roche, Switzerland. *IR* spectra were run on a Perkin Elmer 621 spectrophotometer using KBr pills. Light absorption spectra were recorded with a Varian Cary 118 C spectrophotometer. A Varian EM 390 or a Bruker HFX-90 spectrometer (both 90 MHz)

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were applied for NMR spectroscopy. Chemical shifts refer to C^2HCl_3 ($\delta = 7.24$ ppm) as internal standard and are given in ppm. Coupling constants are given in Hz. Mass spectra were obtained using a Varian CH 7 MAT spectrometer at 70 eV. For thin-layer chromatography, silica gel plastic sheets with an internal fluorescence indicator in the following solvents were used: A: Toluene, B: ethyl acetate, C: dichloromethane/ethanol (10:1), D: ethylacetate/ethanol (1:1), E: toluene/ethyl acetate (1:1), F: hexane/ethyl acetate (10:1), and H: hexane/diethylether (20:1).

Purification and separation of the products were achieved by liquid chromatography on silica gel 60 (70–230 mesh, ASTM) with one of the above-listed solvent systems.

Lumiflavin (*5a*), 3-methyllumiflavin (*5b*), tetraacetylriboflavin (*5d*), and N,3,4-trimethyl-6-(*p*-carboxyphenylazo) aniline (*6*) were synthesized according to Hemmerich et al. [12]. 10-Methyl-isoalloxazine (*5e*) was prepared according to a method described by Kuhn and Weygand [21] and 1,2-dinitro-4,5-dimethylbenzene (*2*) according to Kuhn and van Klaveren [20].

3-Methyl-4-Octadecylaniline

137 g (1.24 moles) *m*-toluidine (*1*), 332 g (1.24 moles) octadecanol, and 85 g (0.62 moles) dry zinc chloride were heated at 240–250 °C for 36 hr. The mixture was then boiled with 500 ml of an 8 N sodium hydroxide solution until the zinc chloride is decomposed. The oily phase was separated and an excess of *m*-toluidine was removed by subsequent steam distillation. The residue was dried over sodium hydroxide and distilled *in vacuo*; the portion distilling between 200 and 250 °C at 10^{-2} Torr was collected.

Yield: 82 g (0.23 moles = 18.4%). ^{1}H-NMR in C^2HCl_3, δ (ppm): 6.88 (d, $^3J = 9$ Hz, 1H, 5-H), 6.45 (d, $^3J = 9$ Hz, 1H, 6-H), 6.47 (s, 1H, 2-H), 3.43 (s, br, 2H, 1-NH_2), 2.45 (t, $^3J = 7.5$ Hz, 2H, 4-CH_2), 2.2 (s, 3H, 3-CH_3), 1.23 (s, 32H, aliphatic protons), 0.87 (t, $^3J = 5.9$ Hz, 3H, terminal CH_3).

3-Methyl-4-Octadecylacetanilide

2 g (5.6 mmol) 3-methyl-4-octadecylaniline were dissolved in 10 ml glacial acetic acid and 5 ml acetic acid anhydride (53 mmol) were added dropwise for 30 min to the hot solution. After 2 hr, the solution was poured into 250 ml water containing 5% sodium hydrogencarbonate. The precipitate was extracted twice with 100 ml ether and the combined organic solutions were dried over sodium sulfate and evaporated to dryness. The residue was dissolved in a minimum of hexane and filtered through charcoal. Cooling gave 0.65 g (1.62 mmol) (29%) of a white powder, which was dried 12 hr over potassium hydroxide at 50 °C and 10^{-2} Torr. mp: 89 °C.

^{1}H-NMR in C^2HCl_3, δ (ppm): 7.17–6.7 (m, 3H, Ar-H), 2.36 (t, $^3J = 8.1$ Hz, 2H, 4-CH_2), 2.08 (s, 3H, 3-CH_3), 1.94 (s, 3H, $COCH_3$), 1.04 (s, 32H, aliphatic protons), 0.73 (t, $^3J = 6$ Hz, terminal CH_3). IR in KBr ν (cm^{-1}): 3290 N-H, 2920, 2850 C-H, 1660 CO.

MS(70 eV, 125 °C) m/e: 401(M^+, 100%), 359(M^+-CH_2O, 5%).

5-Methyl-2-Nitro-4-Octadecylaniline (3a; $R^7 = C_{18}H_{37}$, $R^{10} = H$)

4 g (10 mmol) 3-methyl-4-octadecylacetanilide, dissolved in 25 ml glacial acetic acid anhydride, were added for 10 min to an ice-cold solution of 5 ml acetic acid anhydride and 0.7 ml fuming nitric acid. The temperature was kept between 0 and 5 °C. The mixture was stirred for 3 hr at room temperature until no starting material was detectable by TLC[1] (system F). The reaction mixture was poured in 100 g ice and stirred for 1 hr. The precipitate was filtered, dissolved in hot methanol, and filtered through charcoal. Cooling to 0 °C gave an orange solid. 3.5 g of the solid were dissolved in 50 ml hot ethanol, and 30 ml concentrated hydrochloric acid were added dropwise to this solution and heated for 20 hr. After cooling, the mixture was neutralized with sodium carbonate, extracted with diethylether, and the organic phase was concentrated to a small volume.

The crude product containing two mono-nitro-isomers and a dinitro derivative was separated by column chromatography on silica gel with toluene. The fast fractions contained 0.63 g (1.4 mmol, 14%) 2,6-dinitro-5-methyl-4-octadecylaniline; the later fractions consisted of 1.44 g (3.56 mmol, 35.6%) mono-nitro-isomeric compounds.

5-Methyl-2-Nitro-4-Octadecylaniline: mp: 93 °C

^{1}H-NMR in C^2HCl_3, δ(ppm): 7.8 (s, 1H, 3-H), 6.55 (s, 1H, 6-H), 5.8 (s, br, 2H, 1-NH_2), 2.47 (t, $^3J = 5$ Hz, 4-CH_2), 2.21 (s, 3H, 5-CH_3), 1.23 (s, 32H, aliphatic H's), 0.84 (t, $^3J = 5.6$ Hz, terminal CH_3). MS (70 eV, 100 °C) m/e: 404 (M^+, 100%), 387 ($M^+ - OH$, 23%), 166 ($M^+ - C_{17}H_{34}$, 97%).

IR in KBr ν (cm^{-1}): 3480, 3360 N–H, 2920, 2850 C–H, 1650 aromatic ring, 1500 asymmetric N–O.

2-Nitro-3-Methyl-4-Octadecylaniline: mp: 82 °C

^{1}H-NMR in C^2HCl_2, δ(ppm): 7.02 (d, $^3J = 8.5$ Hz, 1H, 5-H), 6.57 (d, $^3J = 8.5$ Hz, 1H, 6-H), 4.5 (s, 2H, 1-NH_2), 2.5 (t, $^3J = 7.4$ Hz, 2H, 4-CH_2), 2.26 (s, 3H, 3-CH_3), 1.23 (s, 32H, aliphatic H's), 0.86 (t, $^3J = 5.9$ Hz, terminal CH_3).

IR in KBr ν (cm^{-1}): 3440, 3360 N–H, 2920, 2850 C–H, 1635 aromatic ring, 1530 asymmetric N–O, 1355 symmetric N–O. MS (70 eV, 100 °C) m/e: 404 (M^+, 100%), 387 ($M^+ - OH$, 14%), 166 ($M^+ - C_{17}H_{34}$, 43%).

2.6-Dinitro-5-Methyl-4-Octadecylaniline: mp: 102 °C

^{1}H-NMR in C^2HCl_3, δ(ppm): 8.07 (s, 1H, 3-H), 6.62 (s, 2H, 1-NH_2), 2.57 (t, $^3J = 7.5$ Hz, 2H, 4-CH_2), 2.3 (s, 3H, 5-CH_3), 1.24 (s, 32H, aliphatic H's), 0.86 (t, $^3J = 5.9$ Hz, 3H, terminal CH_3).

IR in KBr ν (cm^{-1}): 3470, 3365 N–H, 2920, 2840 C–H, 1630 aromatic ring, 1560, 1535 asymmetric N–O.

MS (70 eV, 175 °C) m/e: 449 (M^+, 91%), 432 ($M^+ - OH$, 100%), 414 ($M^+ - OH$, $-H_2O$, 91%), 211 ($M^+ - C_{17}H_{34}$, 100%), 194 ($M^+ - C_{17}H_{34}$, $-OH$, 38%).

N,5-Dimethyl-4-Octadecyl-2-Nitroaniline (3b, $R^7 = C_{18}H_{37}$, $R^{10} = CH_3$)

0.5 g (1.2 mmol) 5-methyl-4-octadecyl-2-nitroaniline (*3a*) were dissolved in 20 ml methanol and methylated with 0.16 ml (2.6 mmol) methyliodide for 18 hr in an autoclave at 120 °C. The mixture was then evaporated to dryness, dissolved in 100 ml chloroform, washed 3 times with 25 ml 1 N sodium thiosulfate solution and once with 20 ml water. The organic phase was dried over magnesium sulfate, concentrated and purified by column chromatography on silica gel with chloroform/carbon tetrachloride (1:1).

[1] *Abbreviations:* EPR – electron paramagnetic resonance; TLC – thin-layer chromatography; DPL – L-β,γ dipalmitoyl-α-lecithin.

Yield: 0.47 g (1.09 mmol, 91%), mp: 91–92 °C. ^{1}H-NMR in C^2HCl_3, δ (ppm): 7.9 (s, 2H, 3-H, 1-NH), 6.6 (s, 1H, 6-H), 2.98 (d, $^3J=4.5$ Hz, 3H, 1-N-CH_3), 2.49 (t, $^3J=6.5$ Hz, 2H, 4-CH_2), 2.3 (s, 3H, 5-CH_3), 1.26 (s, 32H, aliphatic protons), 0.89 (t, $^3J=6.2$ Hz, 3H, terminal CH_3).

IR in KBr ν (cm^{-1}): 3380 N–H, 2910, 2840 C–H, 1635 aromatic ring, 1570, 1515 asymmetric N–O.

MS (70 eV, 100 °C) m/e: 418 (M^+, 53%), 401 (M^+-OH, 5%), 180 ($M^+-C_{17}H_{34}$, 100%).

7-Octadecyl-3,8,10-Trimethylisoalloxazine (5h)

0.25 g (0.6 mmol) N-methylnitroaniline compound *3b* were dissolved in 250 ml glacial acetic acid and reduced with hydrogen over 250 mg palladium on silica. The crude N-methyl-phenylendiamine solution was filtered in a suspension of 2.5 g boric acid and 300 mg (13 mmol) N-methylalloxane tetrahydrate (*4*) in 50 ml of 90% aqueous acetic acid. After stirring for 15 hr, the mixture was added to a twofold volume of water and filtered. The crude product was recrystallized from ethyl acetate, yielding 0.22 g (0.43 mmol, 71%) 7-amphiflavin (*5h*). mp: 215–218 °C.

Elemental analysis: calculated for $C_{13}H_{48}N_4O_2$ (mol wt 508.75):

C 73.19, H 9.51, N 11.01%

found:

C 72.59, H 9.61, N 10.61%.

^{1}H-NMR in C^2HCl_3, δ (ppm): 8.1 (s, 1H, 6-H), 7.42 (s, 1H, 9-H), 4.12 (s, 3H, 10-CH_3), 3.54 (s, 3H, 3-CH_3), 2.74 (t, $^3J=6$ Hz, 7-CH_2), 2.59 (s, 3H, 8-CH_3), 1.56 (m, 2H, 2′-CH_2), 1.26 (s, 30H, aliphatic protons), 0.88 (t, $^3J=6$ Hz, terminal CH_3).

UV/VIS in ethanol λ [nm] (ε) [M^{-1} cm^{-1}]: 445 (10,900), 352 (8,100), 271 (39,500).

IR in KBr ν (cm^{-1}): 2920, 2850 C–H, 1710 4-CO, 1670 2-CO.

MS (70 eV, 250 °C): m/e 508 (63%, M^+), 494 (100% M^+-CH_2).

Octadecylurea (8)

1 ml Octadecylisocyanate (0.85 g, 2.8 mmol) was dissolved in 10 ml dry ethanol and added dropwise for 20 min to a solution of 25 ml dry ammoniacal ethanol (2 M). The temperature during this procedure was kept at 0 °C. The mixture was allowed to rise to room temperature and was then stirred for a further 12 hr. On adding an equal amount of a 6.5 M aqueous ammonia solution a white solid precipitated, which was filtered by suction. The product was washed with water and cold diethylether and was dried for 12 hr at 10^{-2} Torr over potassium hydroxide.

Yield: 0.73 g (2.3 mmol, 82%) mp: 112 °C. The literature gives 107.8–109 °C [5].

Calculated for $C_{19}H_{40}N_2O$ (mol wt 312.54):

C 73.02, H 12.9, N 8.96%

found:

C 72.99, H 13.01, N 9.10%

^{1}H-NMR in C^2HCl_3, δ (ppm): 4,4 (s, br, 3H, NH, NH_2), 3.06 (t, $^3J=6$ Hz, 2H, N-CH_2), 1.28 (s, br, 32H, aliphatic protons), 0.91 (t, $^3J=5.3$ Hz, 3H, terminal CH_3).

IR in KBr ν (cm^{-1}): 3420, 3340 NH, 2920, 2850 CH, 1650 CO.

MS (70 eV, 250 °C) m/e: 312 (4%, M^+), 269 (13%, M^+–HNCO), 73 (100%, $M^+-C_{17}H_{35}$), 30 (100%, 73–HNCO).

1-Octadecylbarbituric Acid (7)

2.8 ml acetic acid anhydride, diluted with 10 ml glacial acetic acid was added dropwise for 80 min to a stirred solution of 3.1 g (10 mmol) urea *8* and 1.14 g (11 mmol) malonic acid in acetic acid (30 ml) at a temperature of 70 °C. After 30 min the temperature was raised to 90 °C and maintained for 5 hr. The reaction mixture was carefully evaporated under reduced pressure and the residue then refluxed with 50 ml ethanol for 20 min. Cooling yielded 2.77 g of a white product with melting point 102–110 °C.

For elemental analyses and spectroscopic purposes a small amount was dissolved in ethanol/10% concentrated hydrochloric acid and heated for 1 hr. Thereafter, the solution was treated with charcoal filter and the white precipitate recrystallized from ether.

mp: 122–124 °C. Calculated for $C_{22}H_{40}N_2O_3$ (380.57)

C 69.43, H 10.59, N 7.36%

found:

C 69.21, H 10.66, N 7.39%.

^{1}H-NMR in C^2HCl_3, δ (ppm): 7.94 (s, 1H, N–H), 3.83 (t, $^3J=7.4$ Hz, 2H, N-CH_2), 3.62 (s, 2H, CH_2), 1.24 (s, 32H, aliphatic H's), 0.86 (t, $^3J=4.7$ Hz, 3H, terminal CH_3).

IR in KBr ν (cm^{-1}): 2900, 2835 C–H, 1720, 1690, 1670 C=O.

MS (70 eV, 150 °C), m/e: 380 (M^+, 100%).

3-Octadecyllumiflavin (5f)

3.0 g (10.6 mmol) of *p*-(4,5-dimethyl-2-methylaminophenylazo) benzoic acid *6* and 4.56 g (12 mmol) of the barbituric acid *7* were heated for 7 hr in a mixture of 10 ml glacial acetic acid and 40 ml *n*-butanol. The reaction mixture was then evaporated at reduced pressure to dryness and dissolved in 100 ml chloroform. The organic phase was alternately washed with 200 ml water and 200 ml 0.01 M sodium carbonate solution until the water phase remained colorless. Finally, the chloroform phase was washed with 200 ml 0.1 M hydrochloric acid and 200 ml water. The solvent was evaporated under reduced pressure and the residue was dissolved and evaporated to dryness twice with 100 ml toluene each time. The residue was redissolved in a minimum of hot acetic acid and filtered. A few drops of water were added to the clear hot filtrate until a slight precipitate appeared. Storing overnight at 4 °C gave yellow crystals which were filtered by suction, washed with hexane, cold ethanol, hexane and ether, and dried for 12 hr over potassium hydroxide at 100 °C and 10^{-2} Torr.

Yield: 2.6 g (5.1 mmol=48%), mp: 175–190 °C. Calculated for $C_{31}H_{48}N_4O_2$ (508.75):

C 73.19, H 9.51, N 11.01%

found:

C 72.82, H 9.58, N 11.10%.

UV/VIS in ethanol: λ [nm], (ε) [$M^{-1}cm^{-1}$]: 447 (12080), 350 (9400), 271 (40360), 222 (32400).

^{1}H-NMR in C^2HCl_3, δ (ppm): 7.98 (s, 1H, 6-H), 7.33 (s, 1H, 9-H), 4.02 (s, 3H, 10-CH_3), 4.02 (t, $^3J=7.5$ Hz, 2H, 3-CH_2), 2.47 (s, 3H, 8-CH_3), 2.33 (s, 3H, 7-CH_3), 1.63 (br 2H, 2′-CH_2), 1.18 (s, 30H, aliphatic H's), 0.8 (t, $^3J=5.7$ Hz, 3H, terminal CH_3).

IR in KBr ν (cm^{-1}): 2920, 2850 C–H, 1710 4-CO, 1640 2-CO.

MS (70 eV, 250 °C) m/e: 508 (M^+, 50%), 494 (M^+-CH_2, 25%), 213 ($M^+-C_{18}H_{37}NCO$, 100%).

Octadecyl Methanesulfonate

13.5 g (0.05 mol) octadecanol were dissolved in 60 ml pyridine and cooled to below 20 °C. With vigorous stirring 5 ml (0.06 mol) meth-

anesulfonyl chloride was added dropwise. After 4 hr the reaction mixture was poured in 1 liter of 1 M hydrochloric acid and the white solid was collected on a large *Büchner* funnel. After drying in the air the ester was recrystallized from ether/pentane (1:1) yielding 9.65 g. Concentrating of the mother liquid gave a further 3.6 g.

Yield: 13.25 g (0.038 mol, 76%). mp: 60 °C.

^{1}H-NMR in C^2HCl_3, δ (ppm): 4.2 (t, $^3J=6.5$ Hz, 2H, O-CH_2-), 2.95 (s, 3H, S-CH_3), 1.73 (b, 2H, 2-CH_2), 1.23 (s, 30H, aliphatic H's), 0.82 (t, 3H, $J=6.5$ Hz, terminal CH_3).

IR in KBr ν (cm^{-1}): 2920, 2850 aliphatic C–H, 1340, 1330 asymmetric S(=O)$_2$, 1160 symmetric S(=O)$_2$, 982, 945, 850 S–O–C stretch.

MS (70 eV, 125 °C) m/e: 252 ($M^+ - CH_3SO_3H$, 100%).

10-Methyl-3-Octadecylisoalloxazine (5g)

3 g (20 mmol) of powdered dry potassium carbonate were added to a suspension of 0.5 g (2.2 mmol) 10-methylisoalloxazine (*5e*) in 80 ml freshly distilled dimethylformamide. A solution of 1.54 g (4.4 mmol) octadecylmesylate in 50 ml dry dimethylformamide was then added dropwise to the suspension over a period of 1 hr and maintained at 40 °C. After stirring for 4 days, no 10-methylisoalloxazine was detectable by thin-layer chromatography in system B. The reaction mixture was then evaporated to dryness at reduced pressure. The residue was suspended in 200 ml 0.2 M hydrochloric acid and extracted with five portions of 30 ml chloroform. The organic phase was reduced and concentrated to 10 ml and the 3-octadecyl-10-methylisoalloxazine precipitated by addition of five-fold volume of ether/pentane. The yellow solid was filtered, recrystallized from methanol, and dried for 12 hr over potassium hydroxide at 100 °C and 10^{-2} Torr.

Yield: 0.83 g (1.7 mmol=78%). mp: 156–158 °C.

Calculated for $C_{29}H_{44}N_4O_2$ (480.70):

C 72.49, H 9.23, N 11.66%

found:

C 72.18, H 9.35, N 11.53%

^{1}H-NMR in C^2HCl_3, δ (ppm): 0.81 (t, $J=6.0$ Hz, 3H, terminal-CH_3), 1.21 (s, 30H, aliphatic H's), 1.71 (s, b, 2H, 2^1-CH_2), 4.08 (t, $J=7.2$ Hz, 2H, 3-CH_2), 4.1 (s, 3H, 10-CH_3), 7.4–8.0 (m, 3H, ArH's, 7–9), 8.24 (d, $J=7.8$ Hz, 1H, 6-H).

UV/VIS in EtOH, λ [nm], (ε) [M^{-1} cm^{-1}]: 438 (9700), 334 (7920), 268 (37600), 217 (26600).

IR in KBr ν (cm^{-1}): 2900, 2840 C–H long chain; 1700 4-CO, 1638 2-CO.

MS (70 eV, 200 °C) m/e: 480 (M^+, 78%), 466 ($M^+ - CH_2$, 100%) 185 ($M^+ - C_{18}H_{37}NCO$, 30%).

N-Octadecyl-4,5-Dimethyl-2-Nitroaniline (3c, $R^7=CH_3$, $R^{10}=C_{18}H_{37}$)

1 g (5 mmol) 1,2-dinitro-4,5-dimethylbenzene (*2*) and 4 g (14.8 mmol) octadecylamine were heated under an argon atmosphere in 200 ml ethanol. 5 ml of a 1 M sodium carbonate solution were added to the reaction and after 3 hr a further 2 ml were added. After 36 hr no starting material was detectable using TLC in system A, and the mixture was cooled to room temperature and filtered. The residue was dissolved in chloroform, evaporated to dryness, and redissolved in 200 ml diethylether. The etheral solution was washed with 100 ml aqueous acetic acid (5%), 100 ml 0.2 M sodium carbonate solution, and finally with 100 ml half-saturated sodium chloride solution. The organic phase was dried over sodium sulfate, evaporated to dryness, and recrystallized from hexane. The filtrate was evaporated to dryness and then worked off as described before.

Yield: 1 g (2.4 mmol, 48%) mp: 66–67 °C.

^{1}H-NMR in C^2HCl_3, δ (ppm): 7.9 (s, 2H, 3-H, 1-NH), 6.59 (s, 1H, 6-H), 3.25 (q, $^3J=6$ Hz, 2H, 1-NCH_2), 2.24 (s, 3H, 5-CH_3), 2.15 (s, 3H, 4-CH_3), 1.24 (s, 32H, aliphatic protons), 0.86 (t, $^3H=5.5$ Hz, 3H, terminal CH_3).

IR in KBr ν (cm^{-1}): 3370 N–H, 2950, 2920, 2850 aliphatic C-H, 1625 aromatic ring, 1570, 1500 asymmetric N–O.

MS (70 eV, 250 °C) m/e: 418 (100%, M^+), 401 (10%, $M^+ - OH^\cdot$), 390 (90%, $M^+ - C_2H_4$), 373 (8%, $M^+ - C_2H_4, -OH$), 180 (95%, $M^+ - C_{17}H_{34}$), 163 (16%, $M^+ - C_{17}H_{34}, -OH$).

10-Octadecyl-3,7,8-Trimethylisoalloxazine (5i)

0.14 g (3.34×10^{-4} mol) N-octadecyl-4,5-dimethyl-2-nitroaniline (*3c*) were dissolved under warming in 40 ml glacial acetic acid and reduced with hydrogen under atmospheric pressure catalytically with palladium on silica. After 7 hr the reduction was complete and the phenylendiamine solution was filtered into a mixture of 91 mg (4×10^{-4} mol) N-methylalloxane tetrahydrate (*4*) and 100 mg boric acid dissolved in 40 ml of 90% aqueous acetic acid. The reaction was heated for 5 min and stirred a further hour at room temperature. After dilution with 150 ml water, the yellow precipitate was filtered off, washed with water, 5 ml cold diethyl ether, 5 ml cold ethanol and dried 12 hr over potassium hydroxide at 10^{-2} Torr and 80 °C.

Yield: 82 mg (1.6×10^{-4} mol, 48%), mp: 140 °C.

Calculated for $C_{31}H_{48}N_4O_2$ (508.75):

C 73.18, H 9.51, N 11.01%

found:

C 72.42, H 9.50, N 10.64%

^{1}H-NMR in C^2HCl_3, δ (ppm): 8.06 (s, 1H, 6-H), 7.35 (s, 1H, 9-H), 4.67 (t, $^3J=7.2$ Hz, 2H, 10-CH_2), 3.51 (s, 3H, 3-CH_3), 2.53 (s, 3H, 8-CH_3), 2.42 (s, 3H, 7-CH_3), 1.23 (s, 32H, aliphatic protons), 0.85 (t, $^3J=5.4$ Hz, 3H, terminal CH_3 group).

IR in KBr ν (cm^{-1}): 2920, 2850 aliphatic C–H, 1710 4-CO, 1655 2-CO.

MS (70 eV, 225 °C) m/e: 508 (M^+, 100%), 494 ($M^+ - CH_2$, 28%), 480 ($M^+ - CO$, 53%), 451 ($M^+ - CH_3NCO$, 8%), 423 ($M^+ - CH_3NCO$, $-CO$, 8%), 312 ($M^+ - C_{14}H_{28}$, 74%), 284 ($M^+ - C_{16}H_{32}$, 84%).

3-Octadecyllumiflavin-8-carboxylic acid octadecyl ester (9a, $R=C_{18}H_{37}$)

2,3,4,10-Tetrahydro-7,10-dimethyl-3-octadecyl-2,4-dioxo-benzo-[g]pteridine-8-carboxylic acid octadecyl ester

256 mg lumiflavin (*5a*) (1 mmol) and 1.4 g (10 mmol) dried and powdered potassium carbonate were suspended in 100 ml freshly distilled dimethylformamide. A solution of 3.5 g (10 mmol) octadecyl methansulfonate in 100 ml dimethylformamide was added dropwise. The reaction was kept in the dark and was stirred at room temperature for 48 hr until no starting material was detectable, using TLC in system B. The reaction mixture was then worked up as described before. Purification was achieved by column chromatography with System E and precipitation of the product with diethylether.

Yield: 104 mg (0.13 mmol, 13%), mp: 130 °C.

Calculated for $C_{49}H_{82}N_4O_4$ (791.22):

C 74.38, H 10.45, N 7.08%

found:

C 74.27, H 10.48, N 7.03%

^{1}H-NMR in C^2HCl_3, δ (ppm): 8.17 (s, 1H, Ar-H), 8.12 (s, 1H, Ar-H), 4.39 (t, $^3J=6.2$ Hz, 2H, 8αO-CH_2), 4.08 (s, 3H, 10-CH_3), 4.06 (t, $^3J=6.2$ Hz, 2H, 3-CH_2), 2.67 (s, 3H, 7-CH_3), 1.84–1.5 (m, br, 4H, 3αCH_2, 8γCH_2), 1.24 (s, 60H, aliphatic protons), 0.84 (s, br, 6H, terminal CH_3-groups).

UV/VIS in ethanol/chloroform (2:1) λ [nm], (ε) [$M^{-1}cm^{-1}$]: 456 (10000), 338 (10800), 277 (28000), 260 (23600).

IR in KBr ν (cm^{-1}): 2920, 2850 C–H, 1725 8α–CO, 1710 (sh) 4-CO, 1650 2-CO.

MS (70 eV, 300 °C) m/e: 791 (M^+, 25%), 777 (M^+-CH_2, 100%).

3-Methyllumiflavin-8-carboxylic acid octadecyl ester (9b, $R^3=CH_3$, $R=C_{18}H_{37}$)

2,3,4,10-Tetrahydro-3,7,10-trimethyl-2,4-dioxo-benzo[g]pteridine-8-carboxylic acid octadecyl ester

A solution of 1.74 g (5 mmol) octadecylmesylate in 50 ml dry dimethylformamide was added dropwise to a suspension containing 270 mg (1 mmol) 3-methyllumiflavin (*5b*), 1.4 g (10 mmol) powdered potassium carbonate in 50 ml dimethylformamide. The reaction mixture was stirred at room temperature for 48 hr, until no 3-methyllumiflavin was detectable by TLC in ethyl acetate/methanol (20/1). The suspension was then acidified with acetic acid to pH 5 and evaporated to dryness. The residue, suspended in 500 ml water, was extracted with 5 × 200 ml chloroform, dried over sodium sulfate, and evaporated to dryness. Recrystallizing from ethyl acetate gave a yellow powder, which was washed with cold diethylether, yielding 64 mg (0.12 mmol, 12%) 3-methyllumiflavin-8-carboxylic acid octadecyl ester.

mp: 172–175 °C. Calculated for $C_{32}H_{48}N_4O_4$ (552.76):

C 69.53, H 8.75, N 10.14%

found:

C 69.43, H 8.33, N 10.02%

^{1}H-NMR in C^2HCl_3, δ (ppm): 8.1 (s, 1H, Ar-H, 8.14 (s, 1H, Ar-H), 4.38 (t, $^3J=6.3$, 2H, 8αO-CH_2) 4.1 (s, 3H, 10-CH_3), 3.49 (s, 3H, 3-CH_3), 2.68 (s, 3H, 7-CH_3), 1.79 (b, 2H, 8γCH_2), 1.21 (s, b, 30H, aliphatic protons), 0.82 (t, $^3J=6.3$ Hz, 3H terminal CH_3).

UV/VIS in EtOH, λ [nm], (ε) [$M^{-1}cm^{-1}$]: 455 (9890), 335 (10290), 276 (28385).

IR in KBr ν (cm^{-1}): 2920, 2850 C–H long chain, 1725 8α-CO; 1710 4-CO; 1660 2-CO.

MS (70 eV, 200 °C), m/e: 552 (M^+, 100%).

3-Methyllumiflavin-8-carboxylic acid methyl ester (9c, $R^3=R=CH_3$)

2,3,4,10-Tetrahydro-3,7,10-trimethyl-2,4-dioxo-benzo[g]pteridine-8-carboxylic acid methyl ester

0.34 ml (4 mmol) methyl methanesulfonate diluted with 100 ml dimethylformamide were added to a suspension containing 0.1 g (0.4 mmol) 3-methyllumiflavin and 0.55 g (4 mmol) potassium carbonate in 100 ml freshly distilled dimethylformamide. The reaction mixture was stirred at room temperature under exclusion of moisture. TLC in system C showed no further 3-methyllumiflavin after 24 hr. The reaction mixture was acidified with acidic acid and evaporated to dryness. The oily residue was dissolved in 20 ml water and extracted four times with 50 ml chloroform. The combined organic solutions were washed once with 20 ml water dried over magnesium sulfate and then evaporated to dryness. The residue was suspended in diethylether, filtered and recrystallized from methanol.

Yield: 70 mg (55.7%). mp: 276–278 °C.

Calculated for $C_{15}H_{14}N_4O_4$ (314.29)

C 57.32, H 4.49, N 17.83%

found:

C 57.19, H 4.69, N 17.59%.

^{1}H-NMR in CF_3COOH (cation): 8.93 (s, 1H, ArH), 8.56 (s, 1H, ArH), 4.72 (s, 3H, 10-CH_3), 4.27 (s, 3H, 8α–OCH_3), 3.76 (s, 3H, 3-CH_3), 2.93 (s, 3H, 7-CH_3).

IR in KBr ν (cm^{-1}): 2950 aromatic C–H, 1730 8αCO, 1715 4-CO, 1660 2-CO.

^{1}H-NMR in C^2HCl_3 δ (ppm): 8.17 (s, 1H, aromatic H), 8.14 (s, 1H, aromatic H), 4.12 (s, 3H, 10-CH_3), 3.99 (s, 3H, 8α-OCH_3), 3.51 (s, 3H, 3-CH_3), 2.7 (s, 3H, 7-CH_3).

UV/VIS in C_2H_5OH λ [nm], (ε) [$M^{-1}cm^{-1}$]: 454 (9750), 335 (10000), 275 (29000).

MS (70 eV, 200 °C) m/e: 314 (100%, M^+), 257 (44%, M^+-CH_3NCO).

Octadecanol

2.7 g (10 mmol) octadecanol and 0.34 g (1 mmol) tetrabutylammonium hydrogensulfate were dissolved in 50 ml 1,2-dichloroethane and shaken in a separating funnel with 3.3 mmol (0.98 g) sodium dichromate in 25 ml 9 M aqueous sulfuric acid. After separation, the organic phase was washed three times with 20 ml water and then dried over sodium sulfate. The solvent was evaporated at reduced pressure and the residue was dissolved in 20 ml hexane from which a white solid precipitated on standing at −18 °C.

Yield: 0.47 g (1.7 mmol, 17%). mp: 66 °C.

^{1}H-NMR in C^2HCl_3 δ (ppm): 9.75 (s, 1H, 1-H), 2.35 (t, $^3J=6$ Hz, 2H, 2-H), 1.5 (s, br, 2H, 3-H), 1.16 (s, 26H, aliphatic protons), 0.8 (t, $^3J=6.2$ Hz, 3H, terminal CH_3).

5-Octadecyl-1,5-dihydrolumiflavin (10, $R^7=R^8=CH_3$)

1,5-Dihydro-5-octadecyl-3,7,8,10-tetramethyl-benzo[g]pteridine-2,4[3H,10H]-dione

405 mg (1.5 mmol) 3-methyllumiflavin *5b* were dissolved in 500 ml hot acetic acid and reduced under an argon atmosphere with 250 mg zinc powder. An argon flushed solution containing 0.8 g (3 mmol) octadecanal in 150 ml acetic acid was added dropwise to the reaction mixture, maintained at 70 °C. To complete the reduction of the intermediate carbinolamine small amounts (50 mg) of zinc powder were added several times over a period of 5 days. The reaction mixture was then evaporated to a small volume, diluted with 100 ml 2 N perchloric acid, oxidized with sodium nitrite, filtered and dried over phosphorous pentoxide. For further purification, 200 mg of the deep red compound were dissolved in 15 ml chloroform and reduced with 50 ml of an aqueous 10%

acetic acid, containing sodium chloride and sodium dithionite. The solution of the reduced flavin was then chromatographed under anaerobic conditions with system A.

3-Methyllumiflavin-8-carboxylic acid (12a, $R^3 = CH_3$)

2,3,4,10-Tetrahydro-3,7,10-trimethyl-2,4-dioxo-benzo[g]pteridine-8-carboxylic acid

0.5 g (1.85 mmol) 3-methyllumiflavin were dissolved in 200 ml dimethylformamide and after addition of 3 g (21 mmol) powdered potassium carbonate the mixture was stirred at room temperature under aerobic conditions for 50 hr. The mixture was then acidified with 100 ml of a 50% aqueous acetic acid and evaporated to dryness at 40 °C and 1 Torr. The residue was dissolved in the smallest amount of methanol possible. Addition of diethylether gave a yellow solid which was filtered. The solid was dissolved in a small amount of water and precipitated with concentrated hydrochloric acid. The solid was filtered, washed with water and with a small amount of methanol and finally dried over phosphorous pentoxide, 80 °C and 10^{-2} Torr.

Yield: 0.3 g (52%), mp: 302 °C (dec).

Calculated for $C_{14}H_{12}N_4O_4$ (300.27):

C 56.0, H 4.03, N 18.66%

found:

C 56.64, H 3.98, N 18.99%

^{1}H-NMR in ^2H_2O: δ (ppm): 7.84 (s, 2H, ArH), 4.04 (s, 3H, 10-CH_3), 3.32 (s, 3H, 3-CH_3), 2.45 (s, 3H, 7-CH_3).

UV/VIS in 0.1 M phosphate, pH 8.2, λ [nm], (ε) [$M^{-1}cm^{-1}$]: 445 (9000), 361 (7700), 267 (28000).

IR in KBr ν [cm^{-1}]: 3450 8β O–H, 1740 8αC–O, 1700 4 C–O, 1650 2 C–O.

MS (70 eV, 300 °C), m/e: 300 (M^+, 100%), 286 ($M^+ - CO$, 40%), 256 ($M^+ - CO_2$, 61%).

Riboflavin-8-carboxylic acid (12b, $R^3 = H$, $R^{10} = D$-ribityl)

0.75 g (1.37 mmol) tetraacetylriboflavin were dissolved in 250 ml dimethylformamide and after addition of 3.2 g (23 mmol) potassium carbonate stirred under aerobic conditions and exclusion of moisture at room temperature. The reaction was monitored by TLC in system D. After 48 hr no starting material was detectable and the reaction mixture was acidified with acetic acid to pH 5, filtered, and evaporated to dryness at 40 °C and 1 Torr. The residue was redissolved in 150 ml 1-butanol and 1 ml acetic acid to remove inorganic salts. Standing overnight at 0 °C gave 2.4 g of an orange-brown precipitate, which was washed with hexane and diethylether and dried for 18 hr over potassium hydroxide at 100 °C and 10^{-2} Torr. 0.8 g of the precipitate were dissolved in 10 ml methanol, diluted with 40 ml of an ammoniacal methanol, and stirred 50 hr at room temperature. The precipitate was filtered, washed with ether and hexane, and dried for 12 hr at 100 °C over phosphorous pentoxide and 10^{-2} Torr.

Yield: 85 mg (2.1×10^{-4} mol, 46%), mp: 258 °C (dec).

Calculated for $C_{17}H_{18}N_4O_8$ (406.33):

C 50.24, H 4.47, N 13.79%

found:

C 50.09, H 4.54, N 13.63%

^{1}H-NMR in ^2H_2O δ (ppm): 8.03 (s, 1H, ArH), 7.99 (s, 1H, ArH), 2.53 (s, 3H, 7-CH_3).

UV/VIS in 0.1 M phosphate, pH 6.5, λ [nm] (ε) [$M^{-1}cm^{-1}$]: 450 (8500), 364 (6850), 268 (21500).

IR in KBr ν [cm^{-1}]: 3400 O–H, 1715, 1660, 8αC–O, 4 C–O, 2 C–O.

Syntheses

General Remarks

The natural flavin chromophore (Fig. 1) possesses four different chemically active sites [10], namely:

– in the oxidized state the acidic amide group in position 3 and the CH-active methyl group in position 8. Upon deprotonation the latter group becomes oxidizable. Furthermore,

– in the reduced state, the strongly acidic amide region in position 1/2α which reacts under charge control, and the enamine region in position 4a/5 which reacts under frontier orbital control [7]. Thus, we can introduce longchain alkyl residues under oxidized conditions into position 3 and, under reduced conditions, into position 5. The same residues in positions 7 and 10 require new total syntheses of the flavin nucleus.

1) *7-amphiflavins: (Scheme 1)*

The longchain alkyl group was introduced by Friedel Craft's alkylation in position 4 of *m*-toluidine (*1*).

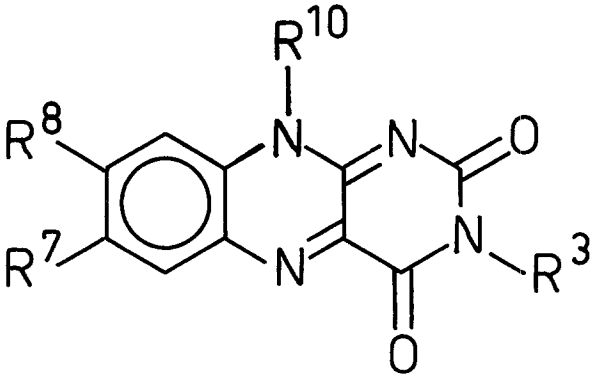
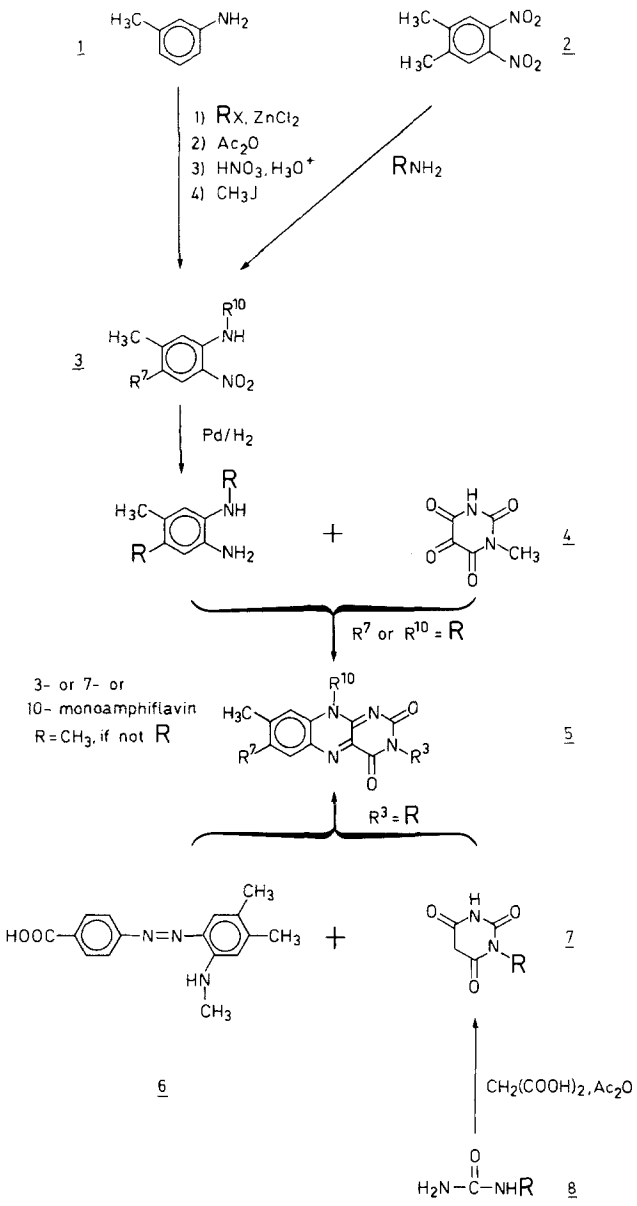


Fig. 1. The natural flavin chromophore: *5a:* lumiflavin $R^3 = H$, $R^7 = R^8 = R^{10} = CH_3$; *5b:* 3-methyllumiflavin $R^3 = R^7 = R^8 R^{10} = CH_3$; *5c:* riboflavin $R^3 = H$, $R^7 = R^8 = CH_3$, $R^{10} = D$-ribityl; *5d:* tetraacetylriboflavin: same as *5c*, but OH groups of D-ribityl are acetylated; *5e:* 10-methylisoalloxazine $R^3 = R^7 = R^8 = H$, $R^{10} = CH_3$; *5f:* 3-amphiflavin $R^3 = C_{18}H_{37}$, $R^7 = R^8 = R^{10} = CH_3$, ("AFl 3", [30]); *5g:* 3-amphiflavin-7,8-bis-nor $R^3 = C_{18}H_{37}$, $R^7 = R^8 = H$, $R^{10} = CH_3$; *5h:* 7-amphiflavin $R^3 = R^8 = R^{10} = CH_3$, $R^7 = C_{18}H_{37}$, ("AFl 7", [30]); *5i:* 10-amphiflavin $R^3 = R^7 = R^8 = CH_3$, $R^{10} = C_{18}H_{37}$, ("AFl 10", [30])



Scheme 1: Preparative pathways for the total syntheses of amphiflavins. $C_{18}H_{37}$-substituents are indicated by bold-faced *R*

After protective acetylation the product underwent *o*-mononitration with 35% yield and to a smaller extent 2,6-dinitration. Following N-deacetylation, the *o*-nitroaniline (*3a*) derivative could be N-monomethylated directly in good yield and was, after catalytic hydrogenation of the nitro group, condensed with N-methylalloxane, yielding 7-amphilumiflavin *5h*. The overall yield of this seven-step synthesis was about 3% based on starting toluidine and 70% based on the more expensive N-methylalloxane [22]. The spectral properties of the new amphiflavin were the same as for the parent compound.

2) *10-amphiflavins: (Scheme 1)*

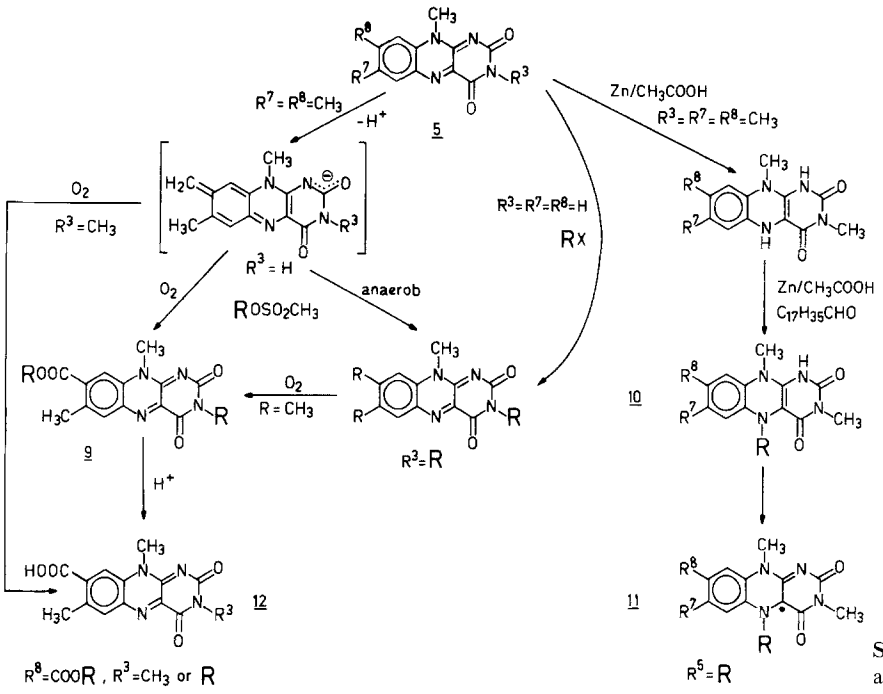
Condensation of *o*-dinitro-xylene (*2*) with primary longchain amines gave the N-amphi-*o*-nitroaniline *3c*, which was catalytically hydrogenated in acetic acid and directly condensed with N-methylalloxane, yielding 23% 10-amphilumiflavin *5i* based on starting dinitro-xylene. The spectral properties of this new flavin were again the same as those of the parent compound.

3) *3-amphiflavins: (Scheme 2)*

Hemmerich [16] has shown that the oxidized flavin nucleus can be selectively alkylated in position 3 by mild base catalysis (potassium carbonate) in dimethylformamide at not too elevated temperatures. With longchain alkylating agents this reaction proved to be much slower and less selective. While position 3 was still the first to react, to our surprise a second longchain alkyl residue was subsequently introduced into the flavin nucleus. Since we suspected that the disturbing second reaction was occurring at the CH-active 8α-center we first omitted the methyl groups in the flavin molecule at positions 7 and 8. With this 7,8-bis-nor-flavin *5e* we obtained selective 3-octadecylation in good yield. However, under strict anaerobic conditions, we observed selective 3-octadecylation with natural lumiflavin *5a* in spite of the 8-methyl group present. The 3-amphiflavin *5f* obtained in this manner was identical with the product of the total synthesis outlined in scheme 1, lower part. For this aim, we synthesized 3-amphiflavin *5f* by means of a Tishler condensation [32] between N-octadecyl-barbituric acid (*7*) and the phenylazo compound *6* [12] in satisfactory yield.

4) *8-amphiflavins: (Scheme 2)*

In order to study what was happening under alkylating aerobic conditions in position 8α, we started with 3-methyllumiflavin (*5b*) and methylmesylate as a strong alkylating agent. Thus, we obtained a new flavin showing a bathochromic shift in the first transition from 446 to 455 nm compared to 3-methyllumiflavin. The fluorescence emission was also shifted bathochromically from 520 to 530 nm. By ^{1}H-NMR a new methyl group was revealed at $\delta = 3.99$ ppm which was incompatible with a C-alkylation. Mass spectroscopy yielded a new parent peak of 314, indicating a gain in mass of 44 units, which is obviously the mass of carbon dioxide. From this we suspected that the 8-methyl group had been attacked by molecular oxygen yielding the 8-carboxylate, which was in



Scheme 2: Alkylation reactions of oxidized and reduced flavins

turn methylated to the methyl ester. Indeed, acid hydrolysis of this new flavin *9c* yielded the authentic chromophore of the known lumiflavin-8-nor-carboxylic acid [15]. After this we confirmed that even in the absence of an alkylating agent, all 8-methyl flavins slowly oxidized in dimethylformamide under aprotic base catalysis (*see* below).

Hence, starting from 3-methyllumiflavin in the presence of octadecyl mesylate we obtained 3-methyllumiflavin-8-carboxylate-amphi-ester *9b*.

5) *3,8-bis-amphiflavins: (Scheme 2, 9a)*

However, when the above reaction was conducted with 3-unsubstituted flavin *5a*, the final product would contain two longchain residues, namely an N-amphi-residue in position 3 and an O-amphi-residue in position 8β, which could be removed by acid hydrolysis.

6) *Flavin-8-carboxylic acids: (Scheme 2, 12)*

As mentioned above, oxidation under aprotic basic conditions lends itself for a smooth synthesis of flavin-8-carboxylic acids, as was shown for the case of riboflavin starting with tetraacetyl riboflavin, which gave by 8α-oxidation and subsequent deacetylation in good yield the useful new flavin derivative riboflavin-8-carboxylic acid (*12b*). The 8-carboxylic acid function was then used, as will be published elsewhere, to create flavin affinity labels for chromatography of flavoapoproteins.

7) *5-amphiflavin: (Scheme 2)*

Starting from reduced flavin, there are two principal routes described for the synthesis of 5-alkylated 1,5-dihydroflavins in the literature.

a) Electrophilic alkylation. The reduced flavin chromophore and its anion possess five centers which should show nucleophilic activity, namely N(5), C(4a), N(1), O(2α) and O(4α). Electrophilic attack in a charge-controlled reaction at the positions N(1), O(2α) and O(4α) was not observed. N(5) and C(4a) are the nucleophilic centers which reacted with electrophilic agents in a frontier-controlled reaction, as shown by Ghisla et al. [7].

b) Electrophilic condensation with aldehydes. Primary and secondary amines can be condensed with formaldehyde to give the corresponding mono- and dimethylated amines after reduction of the carbinolamine with sodium cyanoborohydride [1].

Kemal et al. used this procedure for the reduced flavin as a secondary amine and selectively obtained 5-substituted 1,5-dihydroflavins [19]. The low carbonyl activity of octadecanal, prepared by simple phase transfer catalyzed oxidation of octadecanol with sodium dichromate [26], required intensified reaction conditions. Zinc in refluxing acetic acid is a useful reduc-

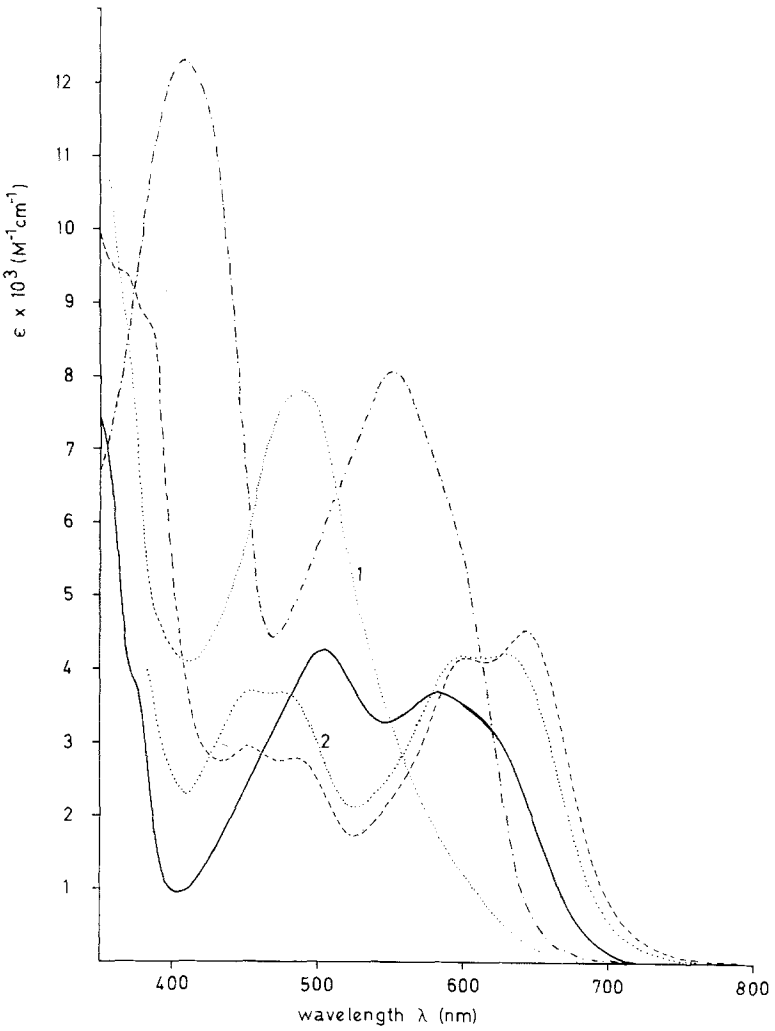
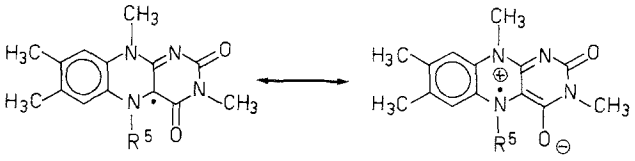


Fig. 2. Absorption spectra of 5-octadecylflavosemiquinone *11* and the corresponding flavinium cation. (–·–·–): 5-octadecylflavinium cation in acetonitrile/2% 6 N perchloric acid, after oxidation with sodium nitrite; (– – – –): 5-octadecylflavosemiquinone *11* ($R^7 = R^8 = CH_3$) in chloroform; (*1* ....): N(1) protonated cation radical *11* in acetonitrile/2% 6 N perchloric acid. (*2* ....): 5-octadecylflavosemiquinone *11* in DPL-vesicles; (——): 5-ethylriboflavinsemiquinone in buffer, pH 4.5/10% dimethylformamide

ing agent for the oxidized flavin [16] and also reduces the intermediate carbinolamine to the 5-octadecylated 1,5-dihydrolumiflavin (*10*). After oxidation of the crude product with sodium nitrite and perchloric acid, we isolated the 3-methyl-5-octadecyllumiflavinium perchlorate as a deep red compound with a long wavelength absorption at 554 nm (*cf.* Fig. 2).

The 5-octadecylflavinium cation can be reduced with sodium dithionite. Upon $1e^-$-oxidation the blue-green neutral flavosemiquinone develops. The absorption spectrum of this compound reveals a considerable influence of the nature of the solvent as shown in Fig. 2. In a polar solvent (water), the long wavelength absorption band is shifted hypsochromically to 580 nm, whereas in strongly unpolar solvents such as chloroform, the n-π^*-absorption occurs at 642 nm. This phenomenon indicates that the ground state of the semiquinone favors the dipolar structure *11b* in both solvents, as explained by Brooker [3]. This structure is also confirmed by EPR studies, which show the largest spin density in position 5 [24]. Incorporation of the semiquinone in artificial phospholipid membranes reveals a broad absorption band at 630 and 600 nm (*cf.* Fig. 2, Curve *2*), exhibiting a hydrophobic environment of the membrane-bound semiquinone.



11 a b

Scheme 3. Uncharged (*a*) and dipolar (*b*) structures of the ground-state flavosemiquinone ($R^5 = C_{18}H_{37}$)

Discussion

Flavin is the unique and ubiquitous redox catalyst which mediates in nature between $1e^-$- and $2e^-$-redox transfer processes [13]. Biological oxidoreduction depends on cofactors which may be roughly subdivided into two classes:

– Catalysts performing activation of specific chemical bonds, such as sp^3-CH-dehydrogenation (nicotinamide cleaving C^+H^- [14], flavin cleaving C^-H^+ [8]), sp^2-CH-dehydrogenation (molybdenum [2]), O_2-activation [18] (heme-Fe [9], Cu [33], flavin, etc.), and

– Catalysts mediating storage, transport, and exchange of single electrons between cell compartments, such as ubiquinone, plastoquinone [23] – all of them showing "detergent" structure by a hydrophilic head and a long lipophilic sidechain.

Quite generally, these head groups are difficult to analyze, especially in the case of benzoquinones, since the various active chromophores occurring in the redox system under physiological conditions (e.g., ubiquinone the species Q_{ox}, $\dot{Q}H$, $\dot{Q}^-$, $Q_{red}H_2$ and $Q_{red}H^-$) cannot be distinguished easily and quantitatively by their spectral properties.

Calvin [4] has pointed out in a recent review the problems in designing chromophoric redox-systems, mimicking this second type of redox transfer catalysts for use in model studies ("synthetic chloroplasts"). He propagates C_{16}-substituted porphyrin and bipyridine chromophores as chelators of suitable redox active metal components. Alternatively, we want to emphasize the use of the well-known natural flavin chromophore as head group for chemical as well as photochemical model studies with artificial membranes [30]. Among the chromophoric species contributing to the flavin system under physiological conditions, Fl_{ox}, $\dot{F}l^-$ and $\dot{F}lH$ can be easily estimated by their optical spectra, while in addition Fl_{ox} can be assayed fluorimetrically and the sum of $\dot{F}l^-$ and $\dot{F}lH$ by EPR. Flavin-loaded artificial membrane systems are thus under investigation in our laboratory, various reports have already been published [6, 29], while a first photochemical approach is described in the accompanying paper [30].

In this context, methods of flavin alkylation, mostly developed in our laboratory, have been generalized for use of long-chain alkyl residues. At this occasion we could elucidate a side reaction, which can give rise to trouble in the N(3)-alkylation of flavins bearing a methyl group in position 8: Under polar aprotic basic conditions, this group is, in principle, reactive, as is well known [10]. If molecular oxygen is present, this methyl group can be oxidized under these conditions to yield the flavin-8-carboxylate, which in turn can be esterified by potent alkylating agents such as methylmethanesulfonate (but not so easily with dimethylsulfate). If selective 3-alkylation is desired, the reaction should thus be kept under inert gas.

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