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On the Chemistry of a Dibenzohypericin Derivative

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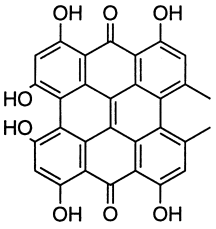
Summary. A hypericin derivative was synthesized in which instead of the methyl groups two benzene rings were condensed to the chromophoric system in order to extend its conjugation. This derivative showed lowered fluorescence and concomitantly enhanced sensitized production of active oxygen species as compared to hypericin. However, in contrast to intuition its long wavelength band remained unshifted in comparison to its parent compound hypericin. Geometry and absorption properties were also investigated by means of semiempirical calculations.

Keywords. Photodynamic therapy; Bilirubin photooxidation; Fluorescence; Hypericin.

Introduction

In the search for better suited photodynamic agents derived from hypericin (**1**), the photosensitizing pigment isolated from the weed St. John's wort (*Hypericum perforatum* L.) [1], a series of derivatives has been synthesized so far to overcome a serious drawback for its use. This has been thought to be necessary because the main absorption band of **1** is situated slightly below 600 nm, which is somewhat outside of the 640–660 nm wavelength of commercial medicinal lasers.



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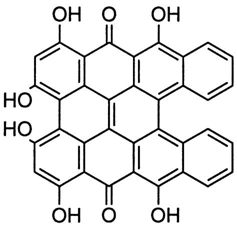

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The efforts to shift the long wavelength absorption band of **1** to longer wavelengths, but concomitantly retaining its main functional groups, centered therefore on a derivatization of **1** with respect to its carbon atoms. Derivatives like 2,5-diodo-hypericin or enhanced conjugation derivatives comprising stilbenoid extension have been synthesized [2, 3]. However, upon irradiation with visible light

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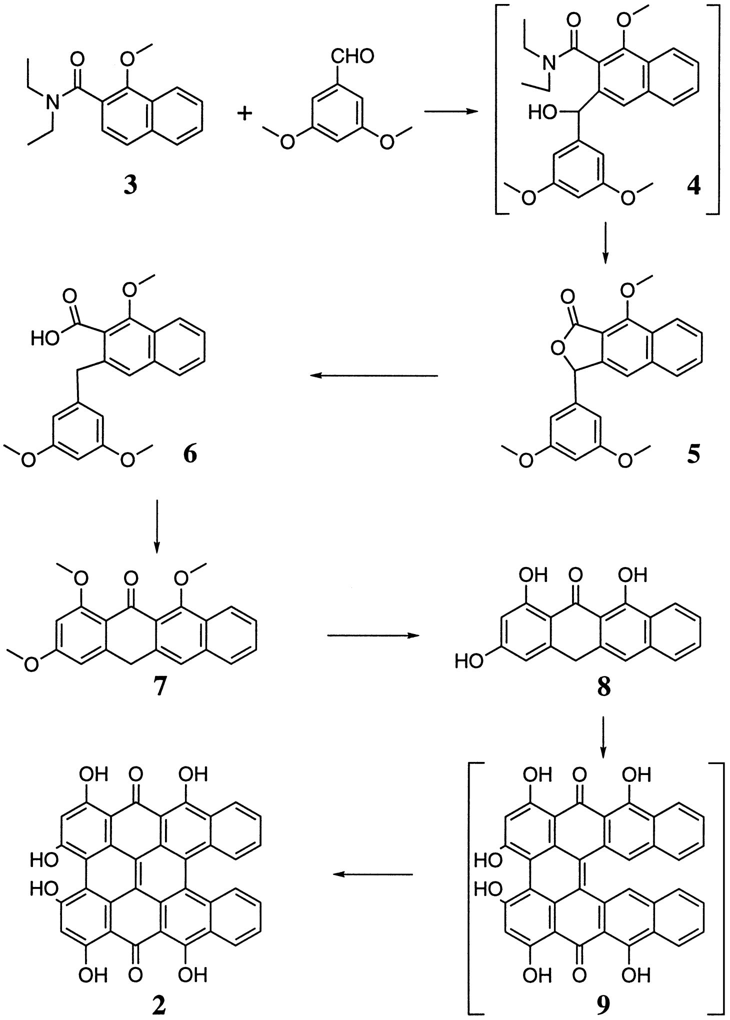
one of the latter rapidly has underwent an intramolecular photochemical [2 + 2] cycloaddition, and upon irradiation the other one did not yield the active oxygen species thought to be essential for its use in photodynamic therapy. This effect has been attributed to interactions of the excited state with its auxochromic amino group. Therefore it was envisaged that extension of the conjugated system and thereby a bathochromic shift of the long wavelength absorption band might be achieved by condensing additional benzene rings to the parent compound **1**. Such a derivative **2** was chosen as the target compound of the present communication.

Results and Discussions

Synthesis

To achieve the goal stated above, the naphthoic acid amide **3** was lithiated with *sec*-butyl lithium, the use of *tert*-butyl lithium having recently been shown to lead to an unprecedented methoxy – *tert*-butyl exchange [4].



Quenching the lithium derivative with 3,5-dimethoxybenzaldehyde yielded **4**. Because of the acid sensitivity of **4** it was immediately converted in about 25% overall yield to the lactone **5** by means of 4-toluenesulfonic acid. Reductive ring opening of **5** with Zn/NaOH provided the naphthoic acid **6** in 47% yield. It should be mentioned that hydrogenolysis on Pd/C resulted in intractable product mixtures in this case. Ring closure of **6** to the tetracene derivative **7** was achieved in high yield by means of trifluoroacetic anhydride in trifluoroacetic acid. Interestingly enough it turned out that this tetracene **7** was present as the carbonyl tautomer instead of the phenolic tautomer as observed in the case of the corresponding anthrone derivative [5]. AM1 calculations revealed that the oxo tautomer **7** was stabilized by $21.5\,\mathrm{kJ \cdot mol^{-1}}$, whereas in the anthrone case the stabilization amounted to $3.5\,\mathrm{kJ \cdot mol^{-1}}$ only. The rather weak stabilization of the latter would be small enough to be overwhelmed by interactions with the solvent and thus account for the observation that in this case the phenol tautomer was the predominant one. Because of the air-sensitivity of compound **7** it was demethylated under reducing conditions by HBr/$SnCl_2$ to give the stable trihydroxytetracenone **8** in high yield. The tetracene derivative **8** was then dimerized in the conventional way of hypericin synthesis [6, 7] to the protohypericin derivative **9**. However, according to an ES-MS and UV/Vis spectrum of the reaction product, most of **9** was already converted to **2** due to its extreme light sensitivity. Completion of the photochemical oxidative ring closure of **9** eventually provided **2** in rather low yield.

Properties

It came as a surprise that the dibenzo-condensed hypericin derivative **2**, which in solution was shown (only one ^{1}H NMR signal of a phenolic proton at position 3/4 at about 18 ppm was observed) to be present as its 3/4 phenolate ion, displayed a long wavelength band at approximately the same wavelength as that observed for the phenolate ion of hypericin (**1**). The overall absorption spectra of the two ions were very similar and, accordingly, the anticipated bathochromic shift of the long wavelength band induced by the conjugation extension of **1** could not be observed. Figure 1 displays the observed spectra of $\mathbf{1}^-$ and $\mathbf{2}^-$ together with the results of the semiempirical PPP-PEP calculations, which recently had been used extensively and successfully on hypericin derivatives [8]. These calculations also showed that, contrary to intuition, one could not assume a significant difference between the absorption spectra of the two compounds. As AM1 calculations showed that the dihedral deformation of the skeletons of **1** in the most stable double butterfly conformations ($\theta_{3,3a,3b,4} = 23.1°$, $\theta_{10,10a,10b,11} = 32.7°$) and **2** ($\theta_{3,3a,3b,4} = 24.6°$, $\theta_{12,12a,12b,12c,12d} = 33.1°$) are virtually identical, the observed and calculated absorption behaviour had to be attributed to the compensation of the loss of the bathochromic effect from the two methyl groups on the one hand and to the small bathochromic effect of the two additional condensed benzene rings on the other hand. Nevertheless, **1** and **2** differed in another aspect relevant to their potential use as photodynamic agents. Thus, $\mathbf{2}^-$ showed a very diminished fluorescence yield ($\Phi_f = 0.03$) as compared to the about ten times higher one of $\mathbf{1}^-$, pointing to an efficient intersystem crossing. Indeed the active oxygen producing efficiency of **2**,

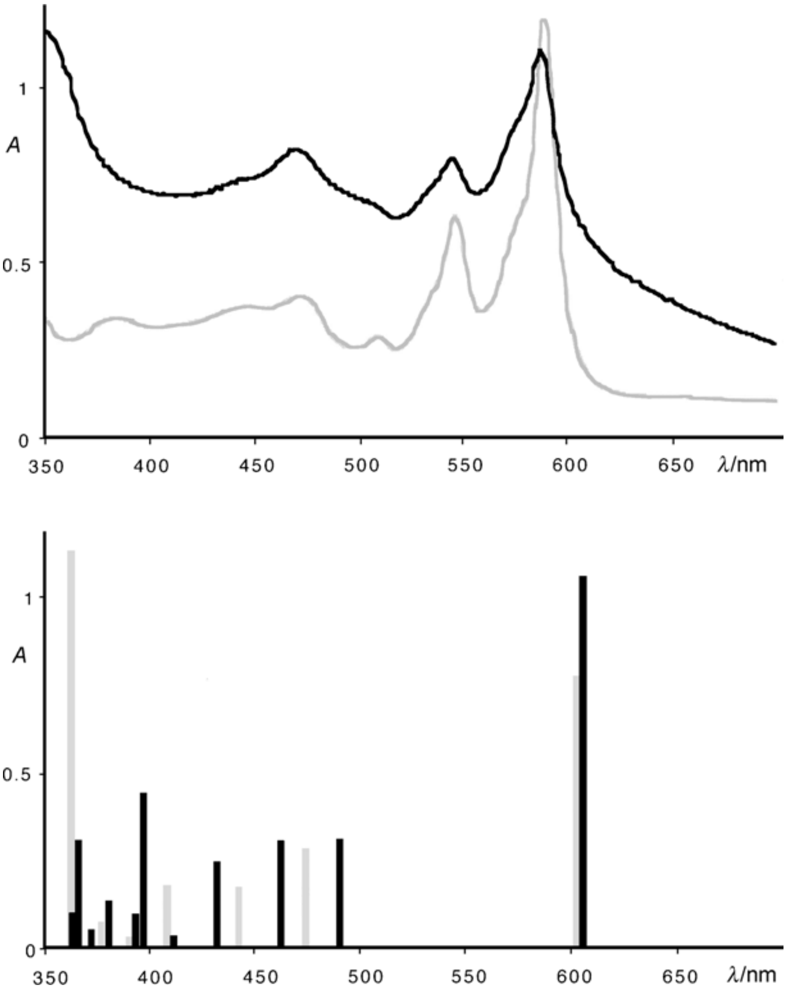


Fig. 1. Absorption spectra of $\mathbf{1}^-$ (grey) and $\mathbf{2}^-$ (black) in MeOH and PPP-PEP calculation results on the same species; the polarization of the absorption bands at *ca.* 600 nm was found to be identical along the C_2-axis of the molecules

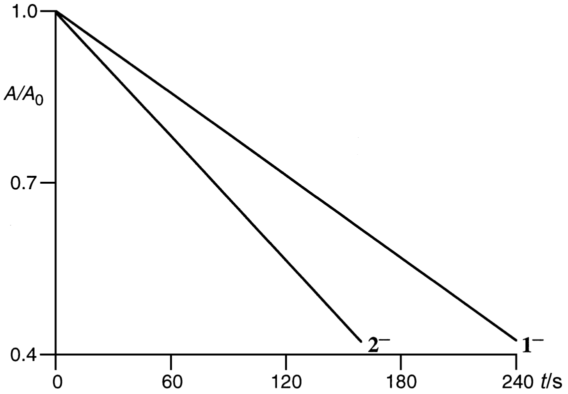


Fig. 2. Hypericin derivative sensitized photooxidation of bilirubin IXα: normalized absorption changes (A/A_0) with time of solutions of disodium bilirubinate IXα together with $^{(3-)}\mathbf{1}$ and $^{(3-)}\mathbf{2}$ in aereated 80% EtOH upon irradiation at $\lambda > 570$ nm

as estimated from the photosensitized destruction of bilirubinate IXα [9], was observed (Fig. 2) to be much more pronounced than that of hypericinate ion ($\mathbf{1}^-$). With respect to the tautomeric equilibria, AM1 calculations revealed that in accordance with the results for **1** [13] the $\mathbf{Q}^{7,14}$ tautomer of **2** is more stable than the most stable $\mathbf{Q}^{1,6}$ tautomer of the remaining tautomers by 90 kJ · mol^{-1}.

In conclusion, it turned out that the dibenzo-condensed derivative of hypericin **2** might well serve as a photodynamic agent of enhanced active oxygen species producing efficiency. Unfortunately, however, it did not yield the envisaged bathochromic shift of its long wavelength absorption band as compared to its parent compound **1** and thus does not convincingly improve the situation with respect to the desired wavelength corresponding to that of medicinal lasers.

Experimental

Solvents were of *p.a.* quality. ^{1}H and ^{13}C NMR, UV/Vis, fluorescence, and mass spectra were recorded using Bruker DRX 500 and DPX 200, Hewlett Packard 8453 UV/Vis, Hitachi 4010F, and Hewlett Packard 59987 quadrupole instruments. The fluorescence quantum yield was determined as described previously [9]. Hypericin sensitized photooxidation of bilirubinate IXα was executed as described in Ref. [10]. AM1 calculations [11] were performed at the SGI Origin 2000 of the LIZENS using the MOPAC package [12, 13]. The PPP-PEP calculations were executed using the PEP program [14]. Substrate **3** was prepared according to Ref. [4]; 3,5-dimethoxy-benzaldehyde was of commercial origin (Aldrich).

1,3,4,6,8,17-Hexahydroxy-benzo[fg]benzo[1′,2′ : 4,5]phenaleno[3,2,1,9-rstuv] pentaphene-7,18-dione (**2**; $C_{36}H_{16}O_8$)

To 67.7 mg **8** (0.23 mmol), 13.5 mg $FeSO_4 \cdot 7H_2O$ (*p.a.*; 0.05 mmol), 155 mg pyridin-N-oxide (1.63 mmol), 1.2 cm^3 absolute pyridine, and 120 mm^3 piperidine were added, and the mixture was heated to 100–110°C under Ar, stirring, and protection from light for 1 h. The reaction mixture was then cooled and poured on 20 cm^3 HCl ($c = 2\,\mathrm{mol \cdot dm^{-3}}$). After standing for 30 min, the dark-blue precipitate was centrifuged and dried over silica. The dry residue consisting mainly of **9** was dissolved in 2.5 dm^3 acetone and irradiated with a 700 W day light lamp (Philips) under stirring and air admission. The bordeaux-red solution was evaporated; the residue was dissolved under sonication in *THF*, centrifuged, and the supernatant was chromatographed on a preparative TLC plate using *THF* : petrol ether : glacial acetic acid = 35 : 5 : 5. The red band was eluted with *THF* : MeOH = 1 : 1. To get rid of the suspended silica, the solution was evaporated and distributed between $CHCl_3$ and H_2O, and the organic phase was evaporated.

Yield: 3.7 mg (2.8%); ^{1}H NMR (200 MHz, δ, *DMSO*-d$_6$): 18.06 (s, 1H, OH-3 or OH-4), 16.20 (s, OH-8,17), 14.14 (s, OH-1,6), 8.65 (d, $J = 6.9$ Hz, H-12,13), 8.18 (X-part of ABCX-system, $J = 7.5$ Hz, H-9,16), 7.90–7.65 (AB or C-part of ABCX-system, H-10,15 or H-11,14), 7.65–7.45 (C or AB-part of ABCX-system, H-11,14 or H-10,15), 6.58 (s, H-2,5) ppm; ^{13}C NMR (125 MHz, δ, *DMSO*-d$_6$): 184.4 (2CO), 166.5 (2COH), 158.0 (2COH), 154.0 (2COH), 133.0, 132.7, 131.6, 129.6, 126.7, 124.3, 124.0, 123.3, 121.1, 117.4, 111.1, 102.2 (28C$_{ar}$) ppm; ES-MS (*i*-PrOH : H_2O = 80 : 20 + 1% NH_3, negative mode): $m/z = 575$ ($(M\text{–}H)^-$); UV/Vis (MeOH, $c = 1 \cdot 10^{-5}\,\mathrm{mol \cdot dm^{-3}}$): $\lambda_{max}(\varepsilon) = 587$ (17850), 545 (12800), 469 (13300), 342 (19500), 290 (36300), 263 (41400), 219 (50400) nm; fluorescence (MeOH, $c = 1 \cdot 10^{-5}\,\mathrm{mol \cdot dm^{-3}}$, $\lambda_{exc} = 550$ nm): λ_{em} (rel. intensity) = 596 (100), 637 (34) nm, $\Phi_f = 0.03$.

(R,S)-N,N-Diethyl-3-((3,5-dimethoxyphenyl)-hydroxymethyl)-1-methoxy-naphthalin-2-carboxamide (**4**; $C_{25}H_{29}NO_5$)

Into a solution of 1.4 cm^3 abs. N,N,N′N′-tetramethylethylenediamine (9.3 mmol) and 1.03 g **3** (4 mmol) in 36 cm^3 absolute. *THF* a solution of *sec*-butyllithium ($c = 1.3\,\mathrm{mol \cdot dm^{-3}}$ in cyclohexane) was added dropwise under Ar at −80 to −85°C during 15 min. To this mixture a solution of 0.73 g 3,5-dimethoxybenzaldehyde (4.4 mmol) in 4 cm^3 absolute. *THF* was added dropwise during 10 min under stirring, and the reaction mixture was stirred for additional 20 min below −80°C. The reaction mixture was then brought to room temperature during 30 min, quenched with 4 cm^3 H_2O, and acidified with 17 cm^3 HCl ($c = 2\,\mathrm{mol \cdot dm^{-3}}$). The organic part of the solvent was evaporated, the residual phase extracted with $CHCl_3$, the organic phase washed with brine, dried over Na_2SO_4, and evaporated. The resulting light-brown oil (2.17 g) was used without further purification for the next step. Column chromatography on Kieselgel 60 using $CHCl_3 : MeOH = 40 : 1$ as the eluent yielded the analytical sample.

R_f (silica, $CHCl_3 : MeOH = 40 : 1$) = 0.36; ^{1}H NMR (500 MHz, δ, $CDCl_3$): 8.09 (d, $J = 8.0$ Hz, H_n-8), 7.75 (d, $J = 7.6$ Hz, H_n-5), 7.55–7.49 (m, H_n-6,7), 7.46 (s, H_n-4), 6.61 (d, $J = 2.2$ Hz, H_b-2 + H_b-6), 6.44 (t, $J = 2.2$ Hz, H_b-4), 5.92 (s, C*H*OH), 3.99 (s, OCH_3), 3.95–3.83 (m, NC*H*H), 3.80 (s, 2OCH_3), 3.45–3.38 (m, NCH*H*), 3.14 (q, $J = 7.2$ Hz, NCH_2), 1.34 (t, $J = 7.1$ Hz, NCH_2CH_3) ppm; ^{13}C NMR (50 MHz, δ, $CDCl_3$): 169.1 (CON), 160.9 (C_n-OMe), 160.8 (2C_b-OMe), 146.4 (C_n), 143.8 (C_n), 140.0 (C_n), 139.6 (C_n), 134.8 (C_n), 128.2 (C_n), 128.7 (C_n), 127.8 (C_n), 126.8 (C_n), 122.6 (C_n), 105.6 (C_b-2,6), 99.3 (C_b-4), 73.1 (CHOH), 62.9 (OCH_3), 55.6 (2OCH_3), 44.0 (NCH_2), 39.1 (NCH_2), 13.1 (NCH_2CH_3), 12.8 (NCH_2CH_3) ppm.

(R,S)-3-(3,5-Dimethoxyphenyl)-9-methoxy-3H-naphtho[2,3-c]furan-1-one (**5**; $C_{21}H_{18}O_5$)

To a solution of 2.17 g crude **4** in 30 cm^3 toluene 10 mg 4-toluenesulfonic acid were added, and the mixture was refluxed for 20 h. After cooling to room temperature the organic layer was washed twice with 5% Na_2CO_3 solution, H_2O, HCl ($c = 2\,\mathrm{mol \cdot dm^{-3}}$), and H_2O. After drying over Na_2SO_4 the solvent was evaporated and the remaining oil crystallized from diethyl ether.

Yield: 0.41 g (25%); m.p.: 135–140°C; R_f (silica, $CHCl_3$) = 0.48; ^{1}H NMR (200 MHz, δ, $CDCl_3$): 8.44 (d, $J = 8.0$ Hz, H_n-8), 7.80 (d, $J = 8.0$ Hz, H_n-5), 7.70–7.45 (m, H_n-6,7), 7.40 (s, H_n-4), 6.51 (d, $J = 2.1$ Hz, H_b-2,6), 6.46 (t, $J = 2.1$ Hz, H_b-4), 6.40 (s, OCH), 4.46 (s, OCH_3), 3.78 (s, 2OCH_3) ppm; ^{13}C NMR (50 MHz, δ, $CDCl_3$): 168.3 (CO), 161.4 (COMe), 158.0 (COMe), 144.8 (COMe), 140.1, 138.1, 129.8, 128.2, 128.1, 126.7, 124.5, 116.2, 110.5, 105.0, 101.0 (13C_{ar}), 82.0 (OCH), 64.2 (OCH_3), 55.7 (2OCH_3) ppm; IR (KBr): $\nu = 3004$, 2946, 2841, 1763, 1633, 1605, 1473, 1462, 1428, 1370, 1346, 1335, 1305, 1286, 1272, 1206, 1164, 1147, 1123, 1089, 1046, 1022, 835 cm^{-1}; UV/Vis ($CHCl_3$, $c = 1 \cdot 10^{-5}\,\mathrm{mol \cdot dm^{-3}}$): $\lambda_{max}(\varepsilon) = 348$ (3840), 303 (4560), 290 (7150), 281 (6720), 246 (50880) nm.

3-(3,5-Dimethoxyphenylmethyl)-1-methoxynaphthalin-2-carboxylic acid (**6**; $C_{21}H_{20}O_5$)

To a solution of 980 mg **5** (2.8 mmol) in 79 cm^3 10% NaOH, 9.95 g activated Zn dust (152 mmol) were added, and the mixture was refluxed for 19 h, cooled on ice, and acidified with 30 cm^3 conc. HCl. After extraction with CH_2Cl_2, washing with brine, and drying over Na_2SO_4 the solvent was evaporated. The residual oil was crystallized from diethyl ether to yield 467 mg (47%) **6**.

M.p.: 147–149°C; ^{1}H NMR (200 MHz, δ, $CDCl_3$): 9.61 (br s, COOH), 8.17–8.09 (m, H_n-8), 7.90–7.65 (m, H_n-5), 7.60–7.48 (H_n-6,7), 7.40 (s, H_n-4), 6.54 (s, H_b-2,6), 6.40 (s, H_b-4), 4.24 (s, CH_2), 4.06 (s, OCH_3), 3.75 (s, 2OCH_3) ppm; ^{13}C NMR (50 MHz, δ, $CDCl_3$): 170.3 (COOH), 161.0, 155.3, 142.4, 136.5, 135.6, 128.2, 126.3, 125.6, 122.8, 121.0, 107.7, 98.5 (16C_{ar}), 64.1 (OCH_3), 55.2 (2OCH_3), 39.9 (CH_2) ppm; IR (KBr): $\nu = 3444$, 2999, 2937, 2841, 2673, 2580, 1686, 1630, 1606, 1595, 1573, 1499, 1454, 1430, 1370, 1353, 1339, 1306, 1292, 1206, 1157, 1144, 1093, 1067, 1056, 954, 946, 922, 843 cm^{-1}; UV/Vis ($CHCl_3$, $c = 1 \cdot 10^{-5}\,mol \cdot dm^{-3}$): $\lambda_{max}(\varepsilon) = 327$ (1700), 283 (7320), 242 (3950) nm; ES-MS ($MeOH : H_2O = 80 : 20 + 1\%$ conc. NH_3, negative mode): *m*/*z* = 351 ($(M\text{–}H)^-$).

5,12-Dihydro-1,3,11-trimethoxytetracen-12-one (**7**; $C_{21}H_{18}O_4$)

Under Ar protection, 27 mg **6** (0.077 mmol) were dissolved in 1 cm^3 trifluoroacetic acid. The solution was cooled to −10°C, and 150 mm^3 trifluoroacetic acid anhydride were added dropwise during 5 min. After stirring for 2 h at −10°C the solution was brought to room temperature and evaporated.

Yield: 23 mg (89%); R_f (silica, $CHCl_3$) = 0.46; ^1H NMR (200 MHz, δ, $CDCl_3$): 8.28 (d, *J* = 8 Hz, H-10), 7.80 (d, *J* = 7.9 Hz, H-7), 7.75–7.35 (m, H-6,8,9), 6.58 (s, H-4), 6.47 (s, H-2), 4.34 (CH_2), 4.17 (s, OCH_3), 3.96 (s, OCH_3), 3.93 (s, OCH_3) ppm; no ^{13}C NMR spectrum could be obtained due to the sensitivity of **7** towards oxidation.

5,12-Dihydro-1,3,11-trihydroxytetracen-12-one (**8**; $C_{18}H_{12}O_4$)

To a solution of 25 mg **7** (0.075 mmol) in 10 cm^3 glacial acetic acid, 0.1 g $SnCl_2 \cdot 2H_2O$ and 1.5 cm^3 47% HBr were added, and the mixture was refluxed for 2 h. The reaction mixture was then poured into 30 cm^3 H_2O and centrifuged. The pellet was washed three times with H_2O and dried over P_4O_{10}.

Yield: 15.3 mg (85%); m.p.: > 320°C; ^1H NMR (200 MHz, δ, *DMSO*-d_6): 13.87 (s, OH-11), 12.39 (s, OH-1), 10.91 (s, OH-3), 8.29 (d, *J* = 8.3 Hz, C-10), 7.85 (d, *J* = 8.1 Hz, H-7), 7.69 (m, H-9), 7.53 (m, H-8), 6.46 (s, H-4), 6.25 (s, H-2), 4.43 (s, CH_2) ppm; ^{13}C NMR (50 MHz, δ, $CDCl_3$): 191.4 (CO), 165.4 (COH), 164.8 (COH), 161.2 (COH), 145.0, 136.5, 134.8, 130.5, 127.0, 125.4, 123.3, 122.7, 116.5, 109.5, 108.5, 107.6, 101.0 ($13C_{ar}$), 32.3 (CH_2) ppm; IR (KBr): $\nu = 1631$, 1597, 1452, 1414, 1398, 1335, 1300, 1161, 1070, 973, 925 cm^{-1}; UV/Vis (*DMSO*, $c = 6 \cdot 10^{-5}\,mol \cdot dm^{-3}$): $\lambda_{max}(\varepsilon) = 403$ (11300), 358 (14900), 312 (10000), 262 (20700) nm.

Acknowledgments

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