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Synthesis and Properties of Fringelite D (1,3,4,6,8,10,11,13-octahydroxy-phenanthro[1,10,9,8, *o*,*p*,*q*,*r*,*a*]perylene-7,14-dione)

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Summary. Fringelite D was synthesized from 1,3,6,8-tetramethoxyanthracen-9-ol *via* two different efficient routes. The first one involved demethylation and subsequent dimerization. The other one started with dimerization to yield octamethylfringelite D and subsequent demethylation. The starting material was prepared in four steps from commercially available educts, the key step being a benzamide *ortho*-lithiation. The spectroscopic properties of fringelite D were measured and are discussed. The dissociation, deprotonation, and protonation equilibria of fringelite D were characterized by their respective *pK* values in ground and excited states and compared with those of hypericin. Homo- and heteroassociation properties of fringelite D were found to be similar to those of hypericin.

Keywords. Fringelite D; Synthesis; Dissociation; Protonation; Deprotonation; Spectroscopic properties; Association.

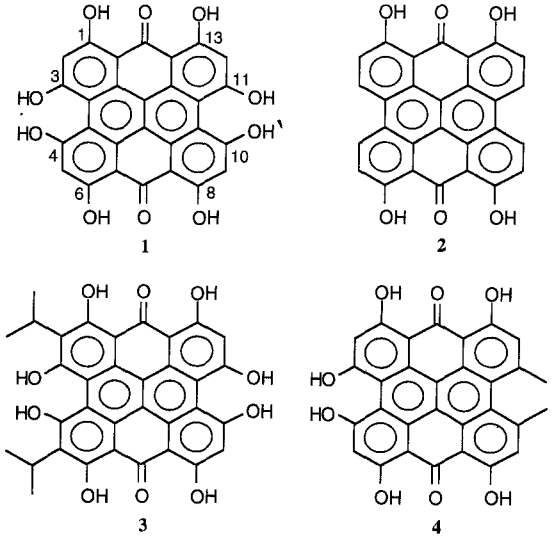
Synthese und Eigenschaften von Fringelit D (1,3,4,6,8,10,11,13-Octahydroxy-phenanthro[1,10,9,8, *o*,*p*,*q*,*r*,*a*]perylen-7,14-dion)

Zusammenfassung. Fringelit D wurde auf zwei effizienten Routen aus 1,3,6,8-Tetramethoxyanthracen-9-ol synthetisiert. Die erste umfaßt Demethylierung und anschließende Dimerisierung. Die andere beginnt mit der Dimerisierung zu Octamethylfringelit D und endet mit einer Demethylierung. Das Ausgangsmaterial wurde in vier Stufen aus kommerziell zugänglichen Edukten dargestellt; der Schlüsselschritt ist eine *ortho*-Lithiierung eines Benzamids. Die spektroskopischen Eigenschaften von Fringelit D wurden gemessen und werden diskutiert. Die Dissoziations-, Deprotonierungs- und Protonierungsgleichgewichte wurden durch ihre *pK*-Werte in Grundzustand und angeregtem Zustand charakterisiert und mit jenen des Hypericins verglichen. Die Homo- und Hetero-assoziationseigenschaften von Fringelit D sind jenen des Hypericins ähnlich.

Introduction

Certain fossil specimens contain red organic pigments, which are termed fringelites. Their structures have been derived to be those of phenanthroperylene quinones substituted with a varying number of hydroxyl groups in positions 1, 3, 4, 6, 8, 10, 11, and 13. The member with the highest number of hydroxyl groups is termed fringelite D (**1**). The fringelites E to G exhibit only 7 to 5 hydroxyl groups, with fringelite H (**2**) as the less substituted derivative [1]. Fringelite related pigments,

called gymnochromes, could be found in recent crinoids [2]. Moreover, the algal pigment stentorin (**3**) [3] and, to a lesser extent, the plant pigment hypericin (**4**) [4] are also related to the fringelite D chromophore.



Besides their importance as fossil constituents these fringelites are of interest in other aspects. They could be used as model compounds for comparisons in the area of hypericin chemistry and physiology. Furthermore, fringelite D (**1**), the chromophoric system of **3**, could serve as a model compound or even as a synthesis intermediate for the study of stentorin chemistry.

Whereas fringelite H is accessible by a synthesis from the "classical" times of hypericin chemistry [5], fringelite D has been prepared only very recently by a rather inconvenient reductive dimerization of 1,3,6,8-tetrahydroxy-9,10-anthraquinone (reaction conditions 120 °C, sealed ampoule, 20 days) [6]. Therefore, a rational convenient synthesis and the chemical and physical properties of fringelite D (**1**), especially in comparison with those of **4**, were thought to be of interest and will be described in this paper.

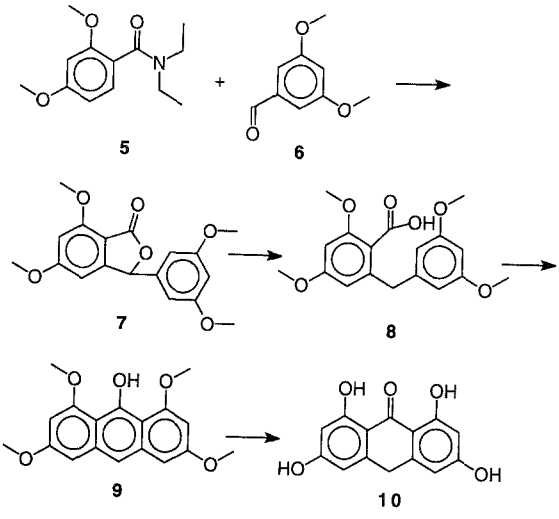
Results and Discussion

Synthesis

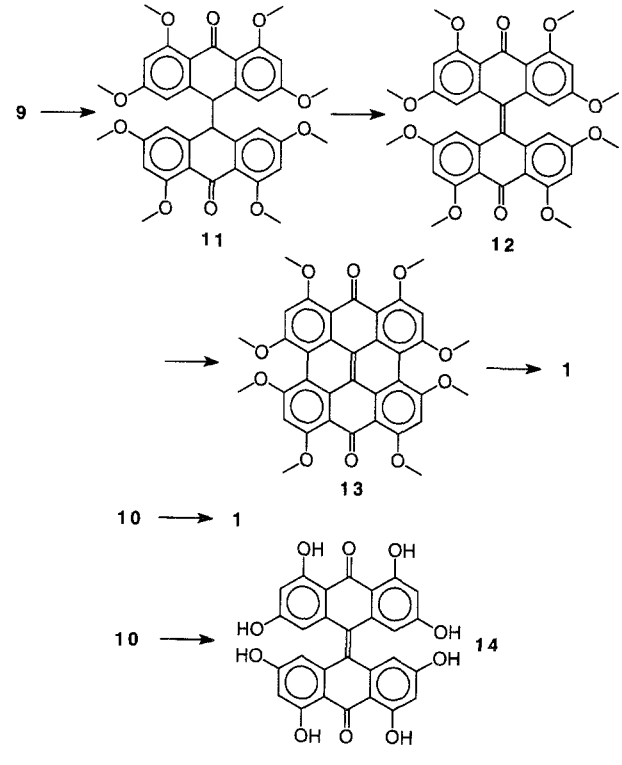
To our experience, the dimerization of anthraquinones to yield phenanthroperylene quinones proved to be inferior to the dimerization of the corresponding anthrones. Therefore, the reduced derivative 1,3,6,8-tetrahydroxy-anthrone was identified as a proper synthon for this reaction.

Starting from the commerically available 2,4-dimethoxybenzoic acid, its diethylamide **5** was prepared according to Ref. [7]. Regioselective lithiation of **5** in the presence of tetramethyl-ethylenediamine according to the procedure of Ref. [8], which uses two moles of *sec*-butyl lithium instead of one [9, 10], yielded after reaction with the commercially available aldehyde **6** the lactone **7**. This lactone was easily hydrogenolyzed over Pd/C to provide the acid **8**. Cyclization of **8** using

trifluoroacetic acid afforded the corresponding tetramethoxy anthrone which was shown to be exclusively present as its anthracenol tautomer **9**. This was derived from the intensity and the chemical shift of its ^{1}H NMR signal of H-10, which was characteristic of one aromatic proton (compare the case of the trimethylemodin anthrone described in Ref. [10]). On demethylation of **9** by means of HJ, the desired anthrone **10** was obtained. The overall yield of **10** in this reaction seqence starting from he amide **5** was 68%.



Scheme 1



Scheme 2

Two variations in the dimerization reaction to yield fringelite D (**1**) were persued. First, a sequence established in a synthesis of hypericin and isohypericin [11] was followed. Starting from the tetramethoxy derivative **9**, the dimerization was achieved by reaction with $FeCl_3$ to afford the bianthraquinoyl derivative **11** in 65% yield. This was then oxidized in alkaline solution using $K_2S_2O_8$ to provide 96% of the bianthrone **12**. After photocyclization of **12** to the otherwise also interesting octamethylfringelite **13**, demethylation with KJ/H_3PO_4 afforded **1** in an overall yield of 15% based on the educt **9**.

The second approach centered on the transformation of **10** to **1**. The tetrahydroxyanthrone **10** was directly reacted with $FeSO_4$ in pyridine in the presence of pyridine-N-oxide and piperidine. This step was followed by photocyclization. The overall yield of the sequence was 48%. The anthrone **10** could also be easily dimerized with $FeCl_3$ to the bianthrone **14** in a 86% yield. However, due to reasons not yet clear, the photocyclization of **14** to yield **1** did not work satisfactorily.

Properties

The constitution of **1** followed from its synthesis and from its NMR spectra. In dimethylsulfoxide as the solvent, the ^1H NMR spectrum displayed one signal at 6.8 ppm for the four aromatic protons in positions 2, 5, 9, and 12. A deuterium exchangeable signal appeared at 14.6 ppm. In analogy to the hypericin (**4**) spectrum [12] it could be assigned to the four hydroxyl groups in the positions adjacent to the carbonyl groups. Upon addition of base, two of the four missing protons resonated at 17.4 ppm. This behavior was similar to that found for **4** [12]. These results were also in agreement with those given in a recent paper in which **1** had been prepared *via* a different route [6]. Moreover, the ^{13}C NMR spectra of **1** and its potassium salt contained the proper number of carbon atom signals which could be assigned and correlated nicely with the corresponding data of **4** [12].

The high symmetry (formal C_{2v}–C_{2h}) of **1** as inferred from its NMR spectra indicated the predominant presence of the 7,14-dioxo tautomer. This has been calculated to be the by far most stable tautomer out of the nine tautomers possible in principle [13].

The absorption spectrum of **1** in dimethylsulfoxide (Fig. 1a) was found to be similar to that of **4**; however, it was slightly shifted to longer wavelengths. Moreover, it displayed a distinct concentration dependence as indicated in Fig. 1a. Whereas at concentrations of 10^{-2} mol/l the long wavelength peak appeared at 601 nm, it was shifted to 614 nm upon dilution of 10^{-7} mol/l. In the intermediate concentration region, the two species gave rise to spectra with isosbestic points. It may be mentioned that a distinct colour change from red to green was observed on proceeding from the concentrated to the dilute solutions. This phenomenon could be described by the linear equation $pK = -3.44\alpha + 4.82$ which yielded a dissociation equilibrium pK value of 3.1 ± 0.4 for pure dimethylsulfoxide. In addition, this phenomenon could be assigned to the dissociation of **1** by a measurement of the molar electrical conductivity. The concentration dependence of the molar conductivity shown in Fig. 1b is characteristic of a dissociation equilibrium with a pK value of about 3 in the case of dimethylsulfoxide.

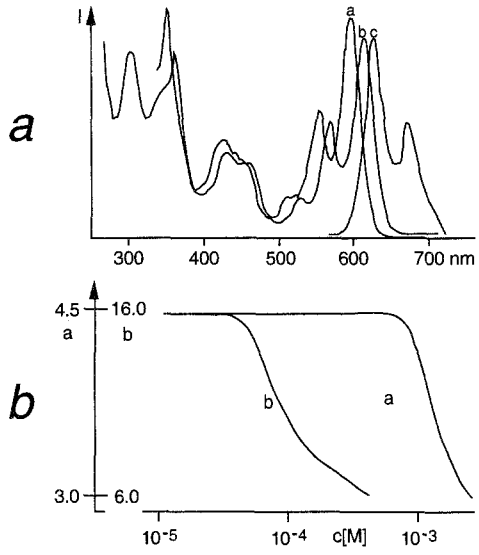
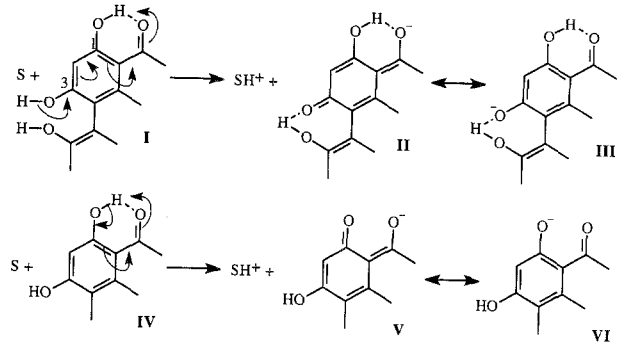


Fig. 1. a Absorption spectra (a: $c = 10^{-2}\,M$ b: $c = 10^{-6}\,M$) and fluorescence spectrum (c: $c = 10^{-6}\,M$) of **1** in dimethylsulfoxide; intensity (I) in arbitrary units; **b** molecular electrical conductivity ($10^{-3} \cdot \mu S \cdot cm^3 \cdot mol^{-1}$) of varying concentrations of **1** in dimethylsulfoxide (a) and ethanol (b)

Accordingly, **1** is a quite strong acid which displays an acidity similar to picric acid. The dissociation takes place at the hydroxyl groups in the bay region as was evident from the ^1H NMR spectra. This behavior may be rationalized from Scheme 3. The *peri*-hydroxyl groups as well as those of the bay region of compounds like **1** are vinylogous carboxylic acids. However, upon deprotonation with the solvent (S) only the anion involving the resonance forms **II** and **III** is efficiently stabilized by mesomerism and hydrogen bonding. In the case of the peri deprotonation, the resonance forms **V** and **VI** become even destabilized by the interaction of the adjacent oxygen lone pairs.



Scheme 3

The *Stokes* shift of the fluorescence of **1** (Fig. 1*a*) was found to be 8 nm, and a fluorescence quantum yield of 0.25 for the dissociated species was estimated. These emission data were similar to those of hypericin (**4**) [12]. The absorption and emission data of the dissociation system of **1** varied slightly for protic and aprotic solvents of different polaritity. Thus, for dilute solutions the long wavelength absorption was observed between the extremal wavelengths of 594 nm (methanol) and 614 nm (pyridine).

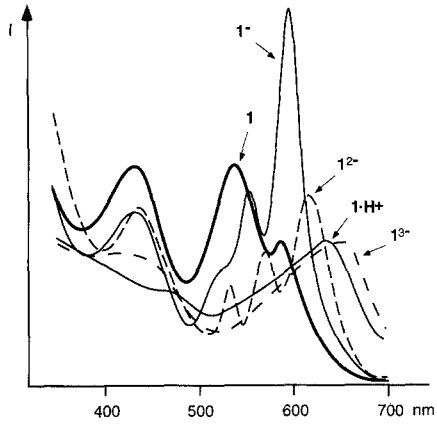


Fig. 2. Absorption spectra (relative intensities) of the neutral (**1**), protonated ($\mathbf{1}\cdot\mathbf{H}^+$), and deprotonated ($\mathbf{1}^-$, $\mathbf{1}^{2-}$, $\mathbf{1}^{3-}$) species of **1** in 80% dimethylsulfoxide/water, 0.011 *M* tetrabutylammonium hydroxide in 50% dimethylsulfoxide/water ($\mathbf{1}^{3-}$), and 85% sulfuric acid ($\mathbf{1}\cdot\mathbf{H}^+$) at concentrations of 10^{-5} *M*

Titration of **1** in aqueous dimethylsulfoxide revealed three deprotonation steps. The absorption spectra of the species **1**, $\mathbf{1}^-$, $\mathbf{1}^{2-}$, and $\mathbf{1}^{3-}$ are shown in Fig. 2 together with those of the protonated system $\mathbf{1}\cdot\mathbf{H}^+$. All species displayed fluorescence, which was characterized by emission wavelengths of 602, 610, 618, 692, and 640 nm and fluorescence quantum yields of 0.10, 0.27, 0.27, 0.02, and 0.21. The deprotonation and protonation behavior of **1** is compared to the data of **4** in Fig. 3. The two deprotonation steps in the bay region of **1** are spaced only slightly more than the statistical factor, indicating that the two hydroxyl groups deprotonated rather independently. One should note that in earlier investigations [12, 14] the strongly acidic step of **4** had escaped our attention, but has been found also recently for aqueous ethanolic solutions [6]. In this respect, tetrahydrofuran seemed to be a distinguished solvent, as the equilibrium between dissociated and undissociated species of **1** developed gradually with time. A similar behavior has been recently found for **4** and was assigned to a tautomeric change [15]. In the light of the above observations on **1** this phenomenon could more conveniently be described by a dissociation equilibrium. It is also suggested that the phenomena described recently with respect to fluorescence life times of **4** [16] could be interpreted on the basis of a dissociation equilibrium.

As illustrated in Fig. 4, $\mathbf{1}^-$ displayed a homoassociation behavior comparable to the one found for **4** [15]. The latter should also be assigned to the dissociated

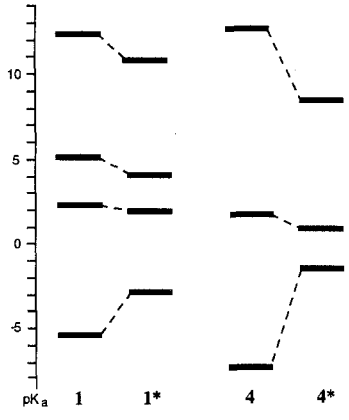


Fig. 3. Protonation and deprotonation pK_a values in ground (**1** and **4**) and excited states (**1*** and **4***)

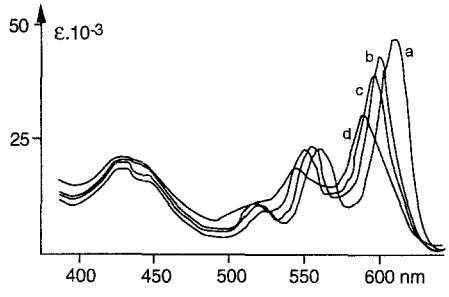


Fig. 4. Absorption spectra of **1** in dimethylsulfoxide water mixtures ($c = 10^{-6}$ *M*); a, 0%; b, 20%; c, 50%; d, 80% water

species. The T_1 values of the protons of **1** in its ^{1}H NMR spectrum were also found to be similar to the values observed for **4** [15].

With respect to heteroassociation it was found that **1** displaced **4** in its complex with human serum albumin. Thus it was bound to the subdomaine *IIIA* [15] more efficiently than **4**. It displayed the absorption and emission characteristics of doubly ionized **1** bound to the protein. Interestingly enough, the human serum albumin complex of **1** did not exhibit a chiroptical signal in the long wavelength band region. This might indicate that the dianion of **1** is in a double butterfly conformation [13] and the chromophore is not intrinsically chiral, but is only chirally disturbed by the protein.

Experimental

Melting points were taken by means of a Kofler hot stage microscope (Reichert, Vienna). ^{1}H, ^{13}C, IR, UV/Vis, and fluorescence spectra were recorded using the Bruker-WM AC-200, Biorad-FT-IR-45, Hitachi-U-3210, and F-4010 instruments. For fluorescence spectroscopy 95% ethanol of "*für die Fluoreszenzspektroskopie*" grade (Merck), otherwise p.a. solvents were used. For the determination of the fluorescence quantum yields (Φ_f) Rhodamine B was used as standard. CD spectra were run on a ISA Mark VI instrument. The pK_a and pK_a^* values of **1** were determined spectrophotometrically using dimethylsulfoxide/water mixtures and tetrabutylammonium hydroxide as the base [17] and by means of *Förster* cycle calculations [18]. A series of aqueous sulfuric acids of known H_0 values [19] was used to determine the protonation pK_a and pK_a^* values. Conductivity measurements (20 °C) were carried out be means of a Metrohm cell ($d = 1$ cm) and the WTW LF 530 apparatus.

Fringelite D (1,3,4,6,8,10,11,13-octahydroxy-phenanthro[1,10,9,8,o,p,q,r,a]perylene-7,14-dione) (**1**; $C_{28}H_{12}O_{10}$)

a) In analogy to the synthesis of **4** according to Ref. [20, 11], 65.6 mg **13** (106 μmol) were dissolved under reflux in 7 ml 85% H_3PO_4. To this mixture three 400 mg portions KJ were added during 1.5 h and the mixture was refluxed for additional 4.5 h. The reaction mixture was brought to room temperature and poured into 80 ml ice water. The resulting precipitate was filtered off, dried, and dissolved in methanol. The filtered solution was evaporated and chromatographed on silica gel with methanol as the eluent. Yield 14.6 mg (25%).

b) In analogy to the synthesis of **4** according to Ref. [21], 102 mg **10** (395 μmol) were dissolved in 2 ml pyridine, and 0.2 ml piperidine, 200 mg pyridine-N-oxide, and 5 mg $FeSO_4 \cdot 7H_2O$ were added. After stirring at 100 °C for 1 h. the cooled solution was acidified with 10 ml 3% HCl. The black precipitate was filtered off, dried, dissolved in 300 ml acetone, and irradiated (mercury pressure lamp) for 20 h. After evaporation of the solvent, the residue was chromatographed on silica gel with methanol as the solvent affording 47 mg. These were then purified by chromatography of its potassium salt on Sepahdex-LH-20 with methanol as the solvent. Yield 25 mg (48%) $\mathbf{1}^-K^+$ as violet to black crystals.

1$^-$K$^+$: m.p.: not up to 320 °C; ^{1}H NMR (*DMSO*-d_6, δ, 200 MHz): 17.41 (s, 2H, OH-3 or OH-4 and OH-10 or OH-11), 14.93 (s, OH-1,6,8,13), 6.59 (s, H-2,5,7,12) ppm; ^{13}C NMR (*DMSO*-d_6, δ, 50 MHz): 183 (C=O), 167.1 (C-3,4,10,11), 165.8 (C-1,6,8,13), 126.7 (C-3a,3b,10a,10b) 121.9 (C-7c,14c), 114.4 (C-6b,7b,13b,14b), 104.9 (C-2,5,9,12), 102.7 (C-6a,7a,13a,14a) ppm. The signals were assigned by an INEPT experiment and by comparison with the data of the hypericin potassium salt [12].

1 (prepared by ether extraction of the potassium salt against 5% HCl): m.p.: not up to 320 °C; ^{1}H NMR (*DMSO*-d_6, δ, 200 MHz, 10^{-2} mol/l): 14.63 (broad s, 4H, OH-1,6,8,13), 6.79 (s, 4H, H-2,5,9,12) ppm; ^{1}H NMR (DMSO-d_6, δ, 200 MHz, $c = 2.4 \cdot 10^{-3}$ *M*): T_1 (14.77 ppm) = 0.94 ± 0.06 s, T_1 (6.71 ppm) = 0.35 ± 0.01 s; ^{1}H NMR (*THF*-d_8, δ, 200 MHz, freshly prepared): 14.24 (s, 4H, OH-1,6,8,13), 6.93 (s, 4 H, H-2,5,9,12) ppm; after 1 d precipitation occurred. After filtration signals were observed at 10.8 (broad s), 8.02 (s), and 7.30 (s) ppm. The residue dissolved in *DMSO*-d_6 gave singlets at 7.94 and 7.36 ppm. ^{13}C NMR (*DMSO*-d_6, δ, 50 MHz): 182.8 (C=O), 167.6 (C-3,4,10,11), 165.8 (C-1,6,8,13), 126.8(C-3a,3b,10a,10b), 122.4 (C-7c,14c), 114.7 (C-6b,7b,13b,14b), 104.9 (C-2,5,9,12), 102.9 (C-6a,7a,13a,14a) ppm. The signals were assigned by an INEPT experiment and by comparison with the data of the hypericin potassium salt [12]. IR (KBr): ν = 1572, 1437, 1419, 1272, 1175, 1110 cm^{-1}; UV/Vis (ethanol, $c = 10^{-5}$ mol/l): λ_{max} = 596 (16400), 552 (7750), 516 (2560), 449 (5060), 389 (14620), 288 (14560) nm (ε); UV/Vis (methanol, $c = 10^{-6}$ mol/l): λ_{max} = 594 (66300), 550 (33900), 511 (16400), 445 (37700) nm (ε); UV/Vis (ethylacetate, $c = 10^{-6}$ mol/l): λ_{max} = 612 (30400), 567 (16900), 525 (8200), 441 (18700) nm (ε); UV/Vis (acetonitrile, $c = 10^{-6}$ mol/l): λ_{max} = 606 (44400), 561 (25000), 526 (11900), 434 (16800) nm (ε); UV/Vis (acetone, $c = 10^{-6}$ mol/l): λ_{max} = 610 (37400), 564 (17900), 525 (7300), 430 (17000) nm (ε); UV/Vis (*DMF*, $c = 10^{-6}$ mol/l): λ_{max} = 613 (52100), 567 (26900), 460 (10300) nm (ε); UV/Vis (*DMSO*, $c = 10^{-5}$ mol/l): λ_{max} = 613 (48800), 567 (21900), 526 (8100), 434 (17100) nm (ε); UV/Vis(pyridine, $c = 10^{-6}$ mol/l): λ_{max} = 614 (20850), 568 (12050), 527 (5620), 434 (9650) nm (ε); UV/Vis (*THF*, immediately after preparation of solution, $c = 5 \cdot 10^{-5}$ mol/l): λ_{max} = 577 (10700), 536 (5400), 501 (2100), 443 (5900), 419 (4300), 334 (10100) nm (ε); UV/Vis (*THF*, after 1 day standing at r.t.): λ_{max} = 609 (26500), 565 (11450), 526 (2600), 448 (18250) nm (ε); fluorescence (methanol, 298 K, λ_{exc} = 550 nm): λ_{em} = 602 (1), 649 (0.30) nm (rel. intensity), Φ_f = 0.30; fluorescence (ethanol, *pH* ≈ 7, 298 K, λ_{exc} = 550 nm): λ_{em} = 605 (1), 650 (0.33) nm (rel. intensity), Φ_f = 0.29; fluorescence (ethylacetate, 298 K, λ_{exc} = 550 nm): λ_{em} = 621 (1), 671 (0.27) nm (rel. intensity), Φ_f = 0.40; fluorescence (acetonitrile, 298 K, λ_{exc} = 550 nm): λ_{em} = 615 (1), 663 (0.29) nm (rel. intensity) , Φ_f = 0.23; fluorescence (acetone, 298 K, λ_{exc} = 550 nm): λ_{em} = 618 (1), 668 (0.26) nm (rel. intensity), Φ_f = 0.37; fluorescence (*DMF*, 298 K, λ_{exc} = 550 nm): λ_{em} = 620 (1), 670 (0.28) nm (rel. intensity), Φ_f = 0.28; fluoresence (*THF*, 298 K, λ_{exc} = 550 nm): λ_{em} = 615 (1), 664 (0.26) nm (rel. intensity), Φ_f = 0.30; fluorescence (pyridine, 298 K, λ_{exc} = 550 nm): λ_{em} = 622 (1), 673 (0.26) nm (rel. intensity), Φ_f = 0.26; fluorescence (*DMSO*, 298 K, λ_{exc} = 550 nm): λ_{em} = 620 (1), 671 (0.25) nm (rel. intensity) Φ_f = 0.27.

pK_a-determinations. Protonation: λ_1 = 596 nm, $\lambda_{1\cdot H^+}$ = 632 nm, $\varepsilon_\lambda/\varepsilon_{\lambda H^+}$ = 0.32; $pK_a(p) = -5.3 \pm 0.2$, $pK_a^*(p) = -3.2 \pm 0.2$. Deprotonation (20% *DMSO*): λ_1 = 594 nm, λ_{1^-} = 605 nm, $\varepsilon_\lambda/\varepsilon_{\lambda^-}$ = 0.62; $pK_a(d^1) = 1.4 \pm 0.05$, $pK_a^*(d^1) = 0.8 \pm 0.05$; $\lambda_{1^{2-}}$ = 621 nm, $\varepsilon_\lambda/\varepsilon_{\lambda^{2-}}$ = 0.98; $pK_a(d_2) = 3.3 \pm 0.2$, $pK_a^*(d_2) = 2.0 \pm 0.2$; $\lambda_{1^{3-}}$ = 637 nm, $\varepsilon_\lambda/\varepsilon_{\lambda^{3-}}$ = 1.09; $pK_a(d_3) = 11.0 \pm 0.2$, $pK_a^*(d_3) = 9.0 \pm 0.2$. Using the water-*DMSO*-tetrabutyl-ammonium hydroxide system [17]: $\lambda_{1^{3-}}$ = 650 nm, $pK_a(d_3) = 12.4 \pm 0.3$, $pK_a^*(d_3) = 10.6 \pm 0.3$. Deprotonation (80% *DMSO*): λ_1 = 593 nm, λ_1 = 598 nm, $\varepsilon_\lambda/\varepsilon_\lambda$ = 0.25; $pK_a(d_1) = 2.2 \pm 0.4$, $pK_a^*(d^1) = 1.9 \pm 0.4$; $\lambda_{1^{2-}}$ = 621 nm, $\varepsilon_\lambda/\varepsilon_{\lambda^{2-}}$ = 1.10; $pK_a(d_2) = 5.1 \pm 0.2$, $pK_a^*(d_2) = 3.9 \pm 0.2$. Fluorescence (80% *DMSO*): **1**: 602, Φ_f = 0.10; **1**$^-$: 610 (1), 651 (0.36), Φ_f = 0.27; **1**$^{2-}$: 618 (1) 662 (0.30) Φ_f = 0.27; **1**$^{3-}$: 692, Φ_f = 0.02 nm (rel. intensity).

The human serum albumin complex of **1** was prepared in analogy to that of **4** [15] in 0.1 *M* phosphate buffer of *pH* 7.0, $c = 1.6 \cdot 10^{-5}$ *M* by mixing solutions of *HSA* (Sigma) and **1**$^-$K$^+$. UV/Vis: 601 (1), 557 (0.71), 517 (0.43), 449 (0.89), 347 (2.57) nm (rel. intensity); fluorescence (excit. 550 nm): 608 (1), 654 (0.30) nm (rel. intensity); CD: 440 (−63), 340 (−37) nm ($\Delta\varepsilon$). Addition of a **1**$^-$K$^+$ solution to the solution of the *HSA*-**4** complex [15] resulted in the complete replacement of the chiroptical signals of **4** by those of the *HSA*-**1** complex.

The following additional data for **4** were also recorded: UV/Vis (80% *DMSO*, *pH* 0.45, **4**): λ_{max} = 586 (2900), 544 (1600), 507 (700), 461 (2000), 320 (3000) nm (ε); fluorescence (excit. 550 nm): 604 (1), 639 (0.34) nm (rel. intensity) $\Phi_f = 0.23$; UV/Vis (80% *DMSO*, *pH* 8.8, $\mathbf{4}^-$): λ_{max} = 599 (3700), 555 (1800), 516 (800), 545 (1300), 388 (1200), 333 (2500) nm (ε); $pK_a = 1.7 \pm 0.2$, $pK_a^* = 0.9 \pm 0.2$; fluorescence (excit. 550 nm): 606 (1), 654 (0.29) nm (rel. intensity) $\Phi_f = 0.23$; fluorescence (excit. 550 nm, 80% *DMSO*, *pH* 13.6, $\mathbf{4}^{2-}$): 618 nm, $\Phi_f = 0.03$.

N,N-Diethyl-2,4-dimethoxybenzamide (**5**)

5 was prepared from commerical 2,4-dimethoxybenzoic acid (Fluka) by conversion to its acid chloride ($SOCl_2$) and reaction with diethyl amine according to Ref. [7] in 40% yield; b.p.: 144–146 °C/0.3 mm Hg.

^{1}H NMR ($CDCl_3$, δ, 200 MHz): 7.00 (d, $J_{5,6}$ = 8.2 Hz, H-6), 6.38 (dd, $J_{5,6}$ = 8.2 Hz, $J_{3,5}$ = 2.1 Hz, H-5), 6.35 (d, $J_{3,5}$ = 2.1 Hz, H-3), 3.69 (s, OCH_3-2 or 4), 3.68 (s, OCH_3-2 or 4), 3.44 (broad m, N–CH_2–CH_3^a), 3.05 (q, J = 7.1 Hz, N–CH_2–CH_3^b), 1.12 (t, J = 7.1 Hz, N–CH_2–CH_3^a); 0.92 (t, J = 7.1 Hz, N–CH_2–CH_3^b) ppm; the assignment of signals was achieved by decoupling experiments. ^{13}C NMR ($CDCl_3$, δ, 50 MHz): 168.3 (C=O), 160.8, 136.1, 119.3 (C-2, C-4, C-1), 127.8 (C-6), 104.2, 98.0 (C-5, C-3), 55.0, 54.9 (2 OCH_3), 42.4, 38.4 (2 CH_2), 13.6, 12.3 (2 CH_3) ppm; the signal assignment was achieved by a ^{1}H-^{13}C-COSY experiment.

5,7-Dimethoxy-3-(3,5-dimethoxyphenyl)-3H-isobenzofuranone (**7**; $C_{18}H_{18}O_6$)

A thoroughly flamed reaction vessel was charged with 25 ml absol. *THF*, 1.18 ml *TMEDA* (Fluka, 7.82 mmol), and 877 mg of **5** (3.70 mmol) dissolved in 5 ml absol. *THF* under an argon atmosphere. After cooling to −80 °C, 6.6 ml of a 1.4 *M sec*- butyl lithium solution (7.84 mmol) were added dropwise within 10 min. After stirring for additional 10 min the solution was colored dark yellow. Within 45 min 1.4 g (8.4 mmol) of 3,5-dimethoxybenzaldehyde (**6**; Aldrich) were added in portions. The solution became first dark orange and, after stirring the reaction mixture for an additional hour, slightly yellow. The reaction mixture was then brought to room temperature, quenched with 30 ml 3 *M* HCl and stirred overnight under an argon atmosphere. The solution was extracted three times with 70 ml $CHCl_3$ and the organic layer washed two times with 70 ml water and with 70 ml sat. NaCl solution. The solvent was then evaporated on a rotatory evaporator and the residue crystallized from toluene/*n*-hexane. The yield was 1.170 g (96%) of white crystals; m.p.: 179–180 °C.

^{1}H NMR ($CDCl_3$, δ, 200 MHz): 6.42 (broad s, H-4,2',4',6'), 6.30 (m, H-6), 6.12 (s, H-3), 3.97 (s, OCH_3-7), 3.81 (s, OCH_3-5), 3.76 (s, OCH_3-3',5') ppm; ^{13}C NMR ($CDCl_3$, δ, 50 MHz): 166.9 (C=O), 161.1 (C-3',5'), 154.4, 139.0, 106.0 (C-7,5,3a,7a), 104.6 (C-2',C-6'), 100.8 (C-4), 99.0 (C-4'), 98.2 (C-6), 81.2 (C-3), 56.0, 55.9 (C-5, OCH_3-7), 55.4 (OCH_3-3'5') ppm. The proton and carbon signals were assigned by means of a ^{1}H-^{13}C-COSY experiment and by comparison with the spectrum of an analogous derivative of Ref. [10]. MS (70 eV, 125 °C): *m/e* (%) = 332 (5, M + 2), 331 (28, M + 1), 330 (92, M), 300 (23), 272 (10), 271 (10), 257 (27), 241 (10), 213 (10), 193 (30), 165 (100), 143 (12), 139 (23), 122 (31), 107 (21), 106 (26), 92 (12), 79 (17), 77 (27), 69 (21), 63 (30), 51 (16); IR (KBr): ν = 1770, 1600, 1500, 1430 cm^{-1}; UV/Vis (ethanol): λ_{max} = 286 (6860) nm (ε).

2,4-Dimethoxy-6-(3,5-dimethoxyphenyl)-methyl)-benzoic acid (**8**; $C_{18}H_{20}O_6$)

660 mg **7** (2 mmol) were dissolved in 50 ml acetic acid. 172 mg Pd(5%)/C was added and the reaction mixture hydrogenated at 1 bar at 65–70 °C for 3 h. The solution was filtered from the catalyst. The catalyst was then washed with hot acetic acid, and the united solutions evaporated on a rotatary evaporator. Crystallization from ethanol/H_2O provided 602 mg (90% yield) of white crystals; m.p.: 140–142 °C.

^{1}H NMR ($CDCl_3$, δ, 200 MHz): 6.39 (d, J = 2.3 Hz, H-3), 6.36 (m, H-2',5,6') 6.29 (m, H-4'), 4.16 (s, CH_2), 3.90 (s, OCH_2-2), 3.76 (s, OCH_3-4), 3.73 (s, OCH_3-3' + OCH_3-5') ppm; ^{1}H NMR (*DMSO*-d_6, δ,

200 MHz): 12.76 (broad s, COOH), 6.46 (d, H-3), 6.37 (m, H-5 + H-2′ + H-6′), 6.30 (d, H-4′), 3.79 (s, CH_2), 3.75 (s, OCH_3-2), 3.72 (s, OCH_3-4), 3.67 (s, OCH_3-3′ + OCH_3-5′) ppm; ^{13}C NMR ($CDCl_3$, δ, 50 MHz): 167.9 (C=O), 160.7 (C-3′,5′), 159.4 (C-6), 162.3, 145.5, (C-1,2,4), 142.6 (C-1′), 108.9 (C-5), 107.2 (C-2′ + C-6′), 98.1 (C-4′), 96.7 (C-3), 56.5 (OCH_3-2), 55.4 (OCH_3-4), 55.2 (OCH_3-3′ + OCH_3-5′), 40.2 (CH_2) ppm; ^{13}C NMR (*DMSO*-d_6, δ, 50 MHz): 168.8 (C=O), 160.3 (C-3′ + C-5′), 157.3 (C-6), 142.5 (C-1′), 160.5, 139.5, 117.6 (C-1 + C-2 + C-4), 106.9 (C-2′ + C-6′), 106.7 (C-5), 97.7 (C-4′), 96.3 (C-3), 55.8 (OCH_3-2), 55.3 (OCH_3-4), 55.0 (OCH_3-3′ + OCH_3-5′), ppm; CH_2-peak covered by *DMSO* signals; IR (KBr): $\nu = 1700, 1600, 1470\,cm^{-1}$; UV/Vis (ethanol: $\lambda_{max} = 282$ (4090), 277 (sh; 3860) nm (ε); MS (70 eV, 110 °C): *m/e* (%) = 334 (7, M + 2), 333 (22, M + 1), 332 (100, M), 314 (46, M-H_2O), 299 (54), 271 (15), 256 (15), 241 (14), 182 (10), 157 (10), 139 (10), 128 (10), 115 (10), 77 (10), 69 (10), 63 (9), 51 (18).

1,3,6,8-Tetramethoxy-anthracen-9-ol (**9**; $C_{18}H_{18}O_5$)

400 mg **8** (1.20 mmol) were dissolved in 16 ml trifluoroacetic acid and cooled to −12 °C. 10 ml of precooled trifluoroacetic acid anhydride were added at once and the mixture stirred for 2 h at −10 °C under an argon atmosphere. The mixture was then evaporated on a rotatory evaporator and the residue crystallized from methanol, resulting in 325 mg (86% yield) of yellow crystals; m.p.: 179–180 °C.

^1H NMR ($CDCl_3$, δ, 200 MHz): 10.68 (broad s, OH), 7.44 (broad s, H-10), 6.61 and 6.31 (2d, $J = 1.8$ Hz, H-2 + 7 and H-4 + 5), 4.02 (s, OCH_3-1 + 8), 3.91 (s, OCH_3-3 + 6) ppm; ^{13}C NMR ($CDCl_3$, δ, 50 MHz): 158.8, 157.8 (C-1 + C-8 and C-3 + C-6), 153.7 (C-9), 136.1 (C-4a + C-10a), 113.2 (C-8a + C-9a), 107.4 (C-10), 96.7, 96.4 (C-2 + C-7 and C-4 + C-5), 56.2, 55.2 (OCH_3-1,8 and OCH_3-3,6) ppm.

1,3,6,8-Tetrahydroxy-anthrone (**10**; $C_{14}H_{10}O_5$)

100 mg **9** (0.32 mmol) were dissolved in 9 ml acetic acid. After addition of 4 ml HJ ($d = 1.70\,g/cm^3$) the mixture was refluxed for 4 h. The reaction mixture was then cooled with ice and after addition of a few drops of water 74.9 mg (91%) slightly yellow microcrystalline product separated, which was isolated by centrifugation; m.p.: 255 °C (dec.).

^1H NMR (*DMSO*-d_6, δ, 200 MHz): 12.46 (s, OH-1 + OH-8), 10.74 (broad s, OH-3 + OH-6), 6.36 and 6.16 (2d, H-2 + H-7 and H-4 + H-5), 4.24 (broad s, CH_2) ppm; ^{13}C NMR (*DMSO*-d_6, δ, 50 MHz): 189.9 (C=O), 164.3 and 164.2 (C-1 + C-8 and C-3 + C-6), 144.4 (C-4a + C-10a), 108.0 (C-8a + C-9a), 107.1 (C-4 + C-5), 100.9 (C-2 + C-7).

1,3,7,9,1′,3′,7′,9′-Octamethoxybianthraquinoyl (**11**; $C_{36}H_{34}O_{10}$)

200 mg **9** (0.64 mmol) were dissolved under reflux in a minimum of ethanol. During 10 min 17.8 ml (0.64 mmol) of a 1% aqueous $FeCl_3$ solution was added dropwise. After refluxing for 45 min the reaction mixture was poured into 800 ml 5% HCl. Following extraction with 300 ml $CHCl_3$ and washings with water and sat. NaCl solution, the solvent was evaporated. After chromatography on 35 g silica gel ($CHCl_3$:methanol = 10:1) the product was crystallized from ethanol to yield 129.1 mg (65%) of faintly rose colored crystals; m.p.: 292–298 °C.

^1H NMR ($CDCl_3$, δ, 200 MHz): 6.38, 5.45 (2d, $J = 2.2$ Hz, 8 H, H-2.2′,7.7′ and H-4,4′,5,5′), 3.90, 3.50 (2s, OCH_3-1,1′,8,8′, H-10,10′, and OCH_3-3,3′,6,6′) ppm; ^{13}C NMR ($CDCl_3$, δ, 50 MHz): 183.1 (C=O), 161.7, 160.5, 144.7 (quartern. C), 104.6, 96.7 (C-2,2′,7,7′ and C-4,4′,5,5′), 59.2 (C-10,10′), 56.1, 55.6 (OCH_3-1,1′,8,8′ and OCH_3-3,3′,6,6′) ppm; IR (KBr): $\nu = 1660, 1600, 1580\,cm^{-1}$; UV/Vis (ethanol): $\lambda_{max} = 324$ (19600), 277 (sh; 18300) nm (ε).

1,3,7,9,1′,3′,7′,9′-Octamethoxybianthrone (**12**; $C_{36}H_{32}O_{10}$)

a) 1.6 g KOH (28.5 mmol) were dissolved in 10 ml $CHCl_3$. After addition of 120 mg **11** (0.19 mmol) the mixture was refluxed for 30 min with exclusion of light. Then this mixture was dropped during 15 min

under vigorous stirring into a solution of 130 mg (0.48 mmol) $K_2S_2O_8$ in 45 ml H_2O. After stirring for 1 h and standing for an additional h at ice bath temperature the product was collected by centrifugation and dried. An additional crop was obtained by extraction of the mother liquid with $CHCl_3$. Yield of raw material 114 mg (96%). To obtain the analytical data a sample was purified by chromatography on silica gel with $CHCl_3$ as the eluent. Due to its sensitivity towards oxygen the raw product was immediately used to prepare **13**.

b) 100 mg **9** (0.32 mmol) were dissolved in a minimum of ethanol. To the refluxing solution 8.6 ml 1% $FeCl_3$ solution (0.32 mmol) were dropped during 30 min. After refluxing for additional 30 min the reaction mixture was poured into 500 ml 5% HCl. Extraction with 250 ml $CHCl_3$ and washing with brine resulted after evaporation in 113.1 mg of yellow brown crystals. 80 mg of this raw material (**11**) were added to a solution of 1 g KOH (17.8 mmol) in 7 ml ethanol. After refluxing for 30 min under exclusion of light, 87 mg $K_2S_2O_8$ dissolved in 28 ml distilled water were added dropwise during 15 min. The reaction mixture was stirred for 1 h and left for another h at 0 °C. The product was centrifuged and washed with water to yield 62 mg yellow-brown crystals (88% based on **9**); m.p.: not up to 320 °C.

^{1}H NMR ($CDCl_3$, δ, 200 MHz): 6.35, 6.12 (2d, 4H, $J = 2.1$ Hz, H-2,2',8.8' and H-4,4',5,5'), 3.88, 3.43 (2s, 12H, OCH_3-3,3',7,7' and OCH_3-1,1',9,9') ppm; IR (KBr): $\nu = 1660, 1600, 1570\,cm^{-1}$; UV/Vis (ethanol): $\lambda_{max} = 374$ (9950), 304 (sh; 18930), 273 (29650) nm (ε).

Octamethylfringelite D (**13**; $C_{36}H_{28}O_{10}$)

50 mg **12** (80 µmol) were dissolved in 150 ml ethanol p.a. and irradiated for 2 h with a low pressure mercury vapor lamp. After evaporation of the solvent and crystallization from $CHCl_3$, 48.2 mg (97%) red crystals were obtained; m.p.: not below 320 °C.

^{1}H NMR ($CDCl_3$, δ, 200 MHz): 7.00 (s, H-2,5,9,12), 4.22, 4.16 (2s, OCH_3-1,3,4,6,8,10,11,13) ppm; IR (KBr): $\nu = 1640, 1590, 1540, 1460\,cm^{-1}$; UV/Vis(ethanol): $\lambda_{max} = 535$ (13430), 502 (8780), 414 (10690), 327 (28300), 290 (30280) nm (ε).

1,3,7,9,1'3',7',9'-Octahydroxybianthrone (**14**; $C_{28}H_{16}O_{10}$)

74.9 mg **10** (0.3 mmol) were dissolved under reflux in 37 ml ethanol p.a. To this solution 67 ml of a 1% ethanolic solution of $FeCl_3 \cdot 6H_2O$ (0.3 mmol) was dropped during 20 min. After refluxing for 4 h the reaction mixture was poured into 500 ml 5% HCl. Extraction with 3 × 100 ml ether, washing with 2 × 100 ml H_2O, 100 ml sat. NaCl, drying over Na_2SO_4, evaporation, and chromatography on silica gel with ethylacetate as the eluent afforded 63.8 mg (86%) of red orange crystals; m.p. 300 °C (dec.).

^{1}H NMR (*DMSO*-d_6, δ, 200 MHz): 12.19, 11.29 (2s, 8H, OH-1,1',9,9' and OH-3,3',7,7'), 7.10, 6.58 (2d, 8H, $J = 2.3$ Hz, H-2,2',8,8' and H-4,4',6,6') ppm; ^{13}C NMR (*DMSO*-d_6, δ, 50 MHz): 181.4 (C=O), 165.0, 164.2, 135.0, 108.7 (5 quart. C), 108.7, 108.1 (C-2,2',8,8' and C-4,4',6,6') ppm; IR (KBr): $\nu = 1620, 1600\,cm^{-1}$; UV/Vis (ethanol): $\lambda_{max} = 456$ (15800), 316 (20800), 293 (46700), 262 (25600) nm (ε).

References

[1] Blumer M. (1951) Mikrochemie **36/37**: 1048; (1965) *Science* **149**: 722; (1962) Geochim. Cosmochim. Acta **26**: 225; (1960) Nature **188**: 1100; (1976) Scientif. Am. **234**: (3) 34; Falk H., Mayr E., Richter A. E. (1994) Microchim. Acta **117**: 1

[2] DeRiccardis F., Iorizzi M., Minale L., Riccio R., Richer de Froges R., Debitus C., (1991) J. Org. Chem. **56**: 6781

[3] Tao N., Orkando M., Hyon J., Gross M., Song P.-S. (1993) J. Am. Chem. Soc. **115**: 2526

[4] Roth L. (1990) Hypericum–Hypericin: Botanik · Inhaltsstoffe · Wirkung. ecomed, Landsberg

[5] Brockmann H., Neeff, R., Mühlmann E. (1950) Chem. Ber. **83**: 467

[6] Freeman D., Frolow F., Kapinus E., Lavie D., Meruelo D., Mazur Y. (1994) J. Chem. Soc. Chem. Commun. **1994**: 891

[7] McCabe E. T., Barthel W. F., Gertler S. I., Hall S. A. (1954) J. Org. Chem. **19**: 493
[8] Townsend C. A., Christensen S. B., Davis S. G. (1988) J. Chem. Soc. Perkin Trans. I **1988**: 839
[9] Mills R. J., Taylor N. J., Snieckus V. (1989) J. Org. Chem. **54**: 4372; Snieckus V. (1990) Chem. Rev. **90**: 879
[10] Falk H., Schoppel G. (1991) Monatsh. Chem. **122**: 739
[11] Falk H., Schoppel G. (1992) Monatsh. Chem. **123**: 931
[12] Falk H., Schmitzberger W. (1992) Monatsh. Chem. **123**: 731
[13] Etzlsdorfer C., Falk H. (1994) Monatsh. Chem. **125**: 955
[14] Falk H., Meyer J., Oberreiter M. (1992) Monatsh. Chem. **123**: 277
[15] Falk H., Meyer J. (1994) Monatsh. Chem. **125**: 753
[16] Gai F., Fehr M. J., Petrich J. W. (1994) J. Phys. Chem. **98**: 5784; Gai F., Fehr M. J., Petrich J. W. (1994) J. Phys. Chem. **98**: 8352
[17] Cox R. A., Stewart R. (1976) J. Am. Chem. Soc. **98**: 488
[18] Ireland J. F., Wyatt P. A. H. (1976) Adv. Phys. Chem. **12**: 131
[19] Paul M. A., Long F. A. (1957) Chem. Rev. **57**: 1
[20] Brockmann H., Kluge F., Muxfeldt H. (1957) Chem. Ber. **90**: 2302
[21] Mazur Y., Bock H., Lavie D. (1991) Can. Pat. Appl. CA 2,029,993

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