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Concerning the Chiral Discrimination and Helix Inversion Barrier in Hypericinates and Hypericin Derivatives

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Summary. It was found that the hypericinate salts of (*R*)-1-phenylethylamine and (*S*)-1-(1-naphthyl)-ethylamine display a small chiroptical signal of the same sign only at high concentrations in an apolar solvent. No further indications of a chiral discrimination between the helical conformers of hypericinate could be found in these cases. However, upon esterification of the 3-hydroxyl group of hypericin with (1*S*)-camphanic chloride, the two diastereomers were found in an 1:1 ratio equilibrating rather fast at temperatures above 30°C with one diastereomer in excess. From the temperature dependence of the equilibrium positions (measured by means of CD and ^{1}H NMR), a ΔG^0 value of $5.8 \pm 0.5\,\mathrm{kJ \cdot mol^{-1}}$ was derived. Accordingly, the chiral discrimination of the (*M*)-configured enantiomer of the helix by the (*S*)-configured auxiliary occurred at an intermediate level. From the temperature dependence of the equilibration kinetics an activation energy of $E_a = 70 \pm 0.5\,\mathrm{kJ \cdot mol^{-1}}$ was derived, which thus defines the upper limit of the helix inversion of hypericin and hypericinate. This value is by about $10\,\mathrm{kJ \cdot mol^{-1}}$ lower than the recently estimated limit.

Keywords. Helix inversion barrier; Diastereomers; Circular dichroism; *Arrhenius* plot; Chirality.

Introduction

The stereochemistry of hypericin (**1**) and its phenolate-type salts is mainly determined by the rather stable helical and thus chiral conformation which the molecule adopts in the solid and crystalline state [1–7]. A certain chiral induction has been observed when **1** was associated to albumin [3], but chiral discrimination and the intrinsic chiroptical properties of the hypericin or hypericinate chromophor have been observed only in the case when one or two (*R*)-menthyl moieties were covalently appended to the methyl groups [5, 6].

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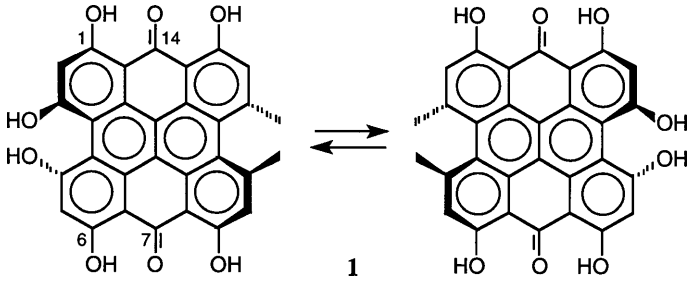

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However, although the chiroptical properties of the hypericin and hypericinate chromophor could be determined this way, the helix inversion barrier obtained for the chromophor might also be influenced partly by the rather sterically demanding residues on the menthyl part. Therefore, it seemed to be desirable to approach the problem by a non-covalent or covalent bond between the oxygen-bearing *bay* region and a chiral auxiliary. Thus, phenolate type salts and an ester at the 3-hydroxy group involving a chiral base or acid were prepared, and their thermodynamic, kinetic, and chiroptical properties were studied.

Results and Discussions

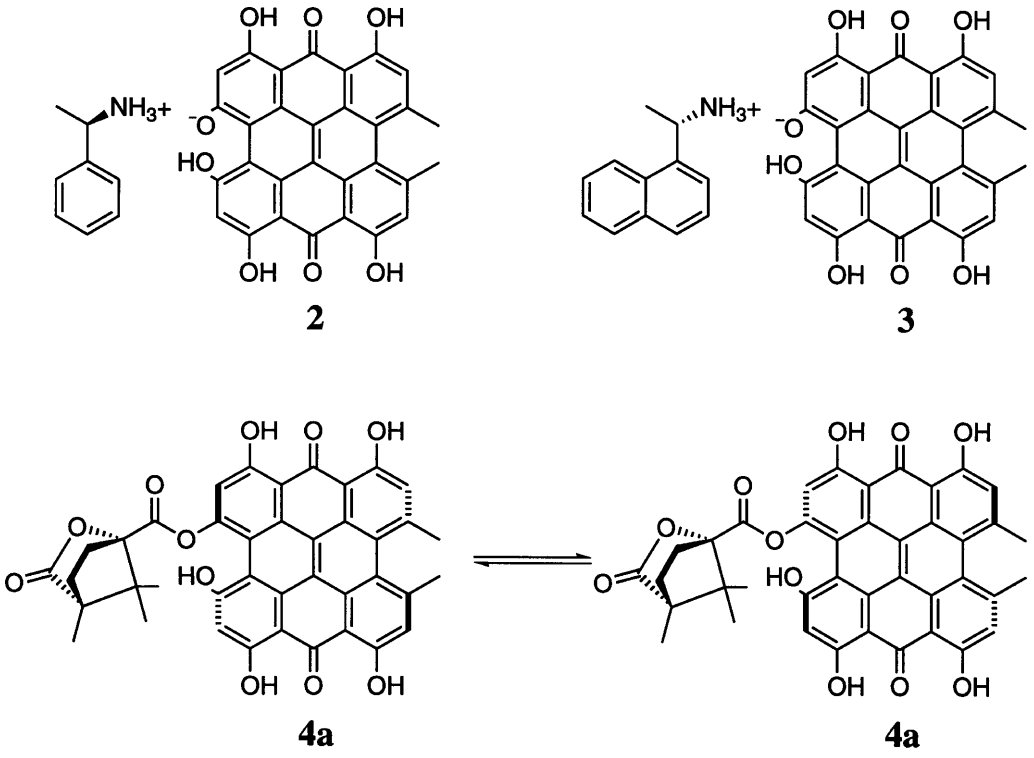
Synthesis

The hypericinate salts of (*R*)-(+)-1-phenylethylamine (**2**), and (*S*)-(−)-1-(1-naphthyl)-ethylamine (**3**), were prepared in the common way of salt formation with hypericin [8]. The hypericin ester of (1*S*)-(−)-camphanic acid at the *bay*-position 3, **4**, was prepared by acylating sodium hypericinate with (1*S*)-(−)-camphanic chloride in *THF* using pyridine to promote the reaction. The spectroscopic properties agreed with the structural hypotheses.

Chiral discrimination

When measuring the chiroptical properties of the diastereomeric salts (*R*, *M*)- and (*R*, *P*)-**2** and (*S*, *M*)- and (*S*, *P*)-**3** it was immediately obvious that they depended mainly upon two parameters: polarity of the solvent and concentration. Thus, with methanol as the solvent no chiroptical signals could be observed even using saturated solutions. In *THF*, the long-wavelength band of the chromophor displayed $\Delta\varepsilon$ values of -6 and -7, which corresponds to a chiral discrimination of about 15% using a $\Delta\varepsilon_0$ value of 40 for this absorption band of the hypericinate chromophore [6]. However, it should be mentioned that the overall shape of the chiroptical dispersion did not perfectly fit that observed for hypericinate [6], and in addition, although the absolute configurations of the two amines used were mirror symmetric, the chiroptical signs of the bands were the same.

The ^{1}H NMR spectra of the two salts in *DMSO*-d_6 showed resonances of the hypericin moiety comparable to those of the sodium salt of hypericin [8] together with the typical signals of the chiral auxiliaries. No significant line broadening was observed. In contrast to this finding, the spectra of **2** and **3** in *THF*-d_8 showed signal groups of two species **2a** + **2b** and **3a** + **3b** with relative intensities of about 1:3. With respect to the nature of the two entities we first thought that these might be



the two respective salt diastereomers. However, the misfit between a chiral discrimination of only 15% evaluated from the chiroptical signal and the relative ^{1}H NMR signal intensities of the two species pointed to the presence of two tautomers in this solvent as another explanation. Accordingly, the question of chiral discrimination and the associated helix inversion barrier height could not be properly addressed by means of the hypericinate salts of chiral auxiliaries.

In the case of the hypericin-3-(1*S*)-camphanate (**4**), the two diastereomers **4a** and **4b** were formed in a ratio of 1:1 as judged from the ^{1}H NMR spectra. Upon equilibration, this ratio became about 1:5. This equilibration became rather fast at temperatures above 30°C, and the thermal stability of the two diastereomers was too low to allow separation and individual characterization. To derive the free energy for this equilibrium, its temperature dependence was measured between 5 and 40°C as shown in Fig. 1. From these data, a value of $\Delta G^0 = 5.8 \pm 0.5\,\mathrm{kJ \cdot mol^{-1}}$ was obtained. Thus, chiral discrimination in this system is of an intermediate level. From the positive sign of $\Delta\varepsilon$ of the long wavelength band of **4b** it followed according to Ref. [6] that in the equilibrium the (*S*, *M*) configured diastereomer **4b** was the favoured one.

Helix inversion barrier

The height of the helix interconversion barrier between the two helix conformers and thus between the two diastereomeric hypericin camphanate esters **4b** and **4b** was estimated from an *Arrhenius* plot of the rate constants of the equilibration conversion of the chromatographically enriched diastereomers **4a** and **4b** (Fig. 2).

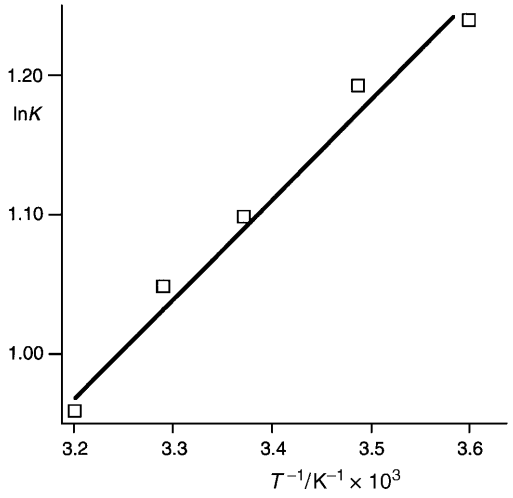


Fig. 1. Temperature dependence of the equilibrium concentrations of diastereomers **4a** and **4b**

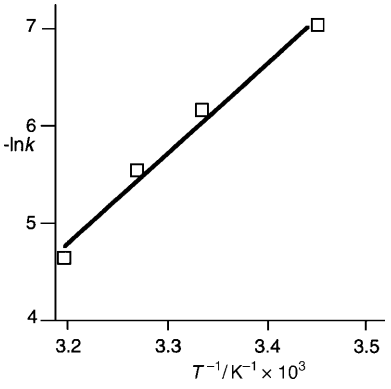


Fig. 2. *Arrhenius* plot for the helix interconversion of diasteromers **4a** and **4b**

It should be noted that the absolute $\pm\,\Delta\varepsilon_0(594)$ values of 40 for the two pure diastereomers obtained from CD measurements and ^{1}H NMR intensities of various mixtures agreed nicely with the value of the hypericin chromophore obtained from the menthyl derivatives [6]. The helix inversion barrier obtained from the *Arrhenius* plot of Fig. 2 amounts to $E_a = 70 \pm 2\,\mathrm{kJ \cdot mol^{-1}}$. This value is somewhat smaller than the barrier extrapolated for hypericin or hypericinate from the interconversion of the ω-menthyl derivative diastereomers ($80\,\mathrm{kJ \cdot mol^{-1}}$). Accordingly, the barrier for the helix interconversion seems to be determined to a small but significant extent by the steric requirements of the appended menthyl residues. The effect of an appendage to the hydroxy groups of the *bay* region should have a smaller, but nevertheless a significant, effect. Thus, we might speculate that the helix inversion barrier of free hypericin or hypericinate in contrast to estimations from ω-appended hypericin derivatives and force field calculations on hypericin ($< 113\,\mathrm{kJ \cdot mol^{-1}}$ [1]) is a little below $70\,\mathrm{kJ \cdot mol^{-1}}$. This also agrees with the fact that for the hypericinate salts with chiral auxiliaries **2** and **3** no indications (line broadenings) for an interconversion process could be observed so far.

Experimental

Melting points were recorded with a Kofler hot stage microscope (Reichert, Vienna). ^{1}H and ^{13}C NMR IR, MS, UV/Vis, and CD measurements were performed on Bruker DRX 500, Biorad FT-IR 45, Fisons MD 800, Hewlett Packard 8453, and Jasco J-810 instruments. For UV/Vis and CD experiments, $CHCl_3$ percolated over Al_2O_3, *DMSO*, *THF*, or MeOH (spectroscopic grade, Merck) were used as solvents; a sample cell of 0.1 mm length was used for the concentration of $2 \cdot 10^{-3}$ mol · dm^{-3}, a cell or 1.0 cm length for the concentration of $2 \cdot 10^{-5}$ mol · dm^{-3}. Hypericin (**1**) was prepared and purified as described [8, 9], its Na salt was synthesized according to Ref. [8]. Concentration data for thermodynamic and kinetic analyses were obtained by measurements of $\Delta\varepsilon$ at 594 nm or by integration of the corresponding *peri*-OH signals of the hypericin chromophore or of the CH_3 signals of the camphane moieties in the ^{1}H NMR spectra of **4a** and **4b** in $CDCl_3$:*DMSO* = 10:03 measured at different temperatures (5, 15, 25, 32, 40°C). The equilibrium constants K were calculated from the concentrations of **4a** and **4b** after equilibrium was reached in each case; ΔG^0 was calculated from the equation $\Delta G^0 = -RT\ln K$. Using the values of the rate constants k at the temperatures given above, the activation energy E_a was calculated from the *Arrhenius* equation $\ln k = -E_a/RT + \ln A$.

(R)-(+)-1-Phenylethylammonium hypericinate (**2**; $C_{38}H_{27}NO_8$)

To a stirred solution of 20 mg **1** (0.04 mmol) in 10 cm^3 MeOH, a solution of 90 mg (*R*)-(+)-1-phenylethylamin (1 mmol) in 2 cm^3 MeOH was added. The reaction mixture was stirred for 1 h at room temperature and then centrifuged. The solution was concentrated *in vacuo* to a violet residue. Washing the residue with hexane : $CHCl_3$ = 5:1 gave 19 mg **2** (80%) as a 1:3 diastereomeric/tautomeric mixture; m.p.: >340°C.

Diastereomer/tautomer **2a**: ^{1}H NMR (*THF*-d_8, δ, 500 MHz, 25°C): 17.65 (s, OH), 14.75 (s, 2OH), 14.17 (s, 2OH), 7.87 – 7.32 (m, 5H-phenyl), 7.35 (s, 2H-ar), 6.64 (s, 2H-ar), 4.69 (bs, CH), 2.78 (s, $2CH_3$), 1.59 (s, CH_3) ppm.

Diastereomer/tautomer **2b**: ^{1}H NMR (*THF*-d_8, δ, 500 MHz, 25°C): 17.65 (s, OH), 14.69 (s, 2OH), 14.10 (s, 2OH), 7.87 – 7.32 (m, 5H-phenyl), 7.35 (s, 2H-ar), 6.64 (s, 2H-ar), 4.52 (s, CH), 2.78 (s, $2CH_3$), 1.63 (s, CH_3) ppm.

Diastereomers/tautomers **2a** + **2b** (1:3): ^{1}H NMR (*DMSO*-d_6, δ, 500 MHz, 25°C): 18.38 (s, OH), 14.2 – 14.8 (bs, 4OH), 7.46 – 7.36 (m, 5H-phenyl), 7.41 (s, 2H-ar), 6.54 (s, 2H-ar), 4.38 (s, CH), 2.72 (s, $2CH_3$), 1.48 (s, CH_3) ppm; UV/Vis (*THF*, $c = 1.5 \cdot 10^{-3}$ mol/dm^3, 25°C): $\lambda_{max}(\varepsilon) = 599$ (30427), 555 (19385), 517 (8815), 481 (12465), 385 (11289), 335 (24779) nm; CD (*THF*, $c = 1.5 \cdot 10^{-3}$ mol/dm^3, 25°C): $\lambda_{max}(\Delta\varepsilon) = 599$ (−6.4), 555 (−2.8), 416 (+1.6), 335 (−1.8) nm; IR (KBr): $\nu = 3432$, 1589, 1555, 1498, 1259 cm^{-1}.

(S)-(−)-1-(1-Naphthyl)-ethylammonium hypericinate (**3**; $C_{42}H_{29}NO_8$)

To a stirred solution of 10 mg **1** (0.02 mmol) in 5 cm^3 MeOH, a solution of 40 mg (*S*)-(−)-1-(1-naphthyl)-ethylamine (0.6 mmol) in 2 cm^3 MeOH was added. The reaction mixture was stirred for 1 h at room temperature and then centrifuged. The solution was concentrated *in vacuo* to a violet residue. Washing the residue with $CHCl_3$:hexane = 2:1 gave 10 mg **3** (72%) as a 1:3 diastereomeric/tautomeric mixture; m.p.: >340°C. In solution at room temperature, the two diastereomers/tautomers equilibrated to a final ratio of 25:75.

Diastereomer/tautomer **3a**: ^{1}H NMR (*THF*-d_8, δ, 500 MHz, 25°C): 17.55 (s, OH), 14.74 (s, 2OH), 14.17 (s, 2OH), 8.02 (d, $J = 8$ Hz, naphthyl-H-2), 7.76 (d, $J = 8$ Hz, naphthyl-H-4), 7.73 (d, $J = 8$ Hz, naphthyl-H-5), 7.67 (d, $J = 8$ Hz, naphthyl-H-8), 7.41 (m, naphthyl-H-3, H-6, H-7), 7.35 (s, 2H-ar), 6.66 (s, 2H-ar), 5.34 (m, CH), 2.79 (s, $2CH_3$), 1.72 (s, CH_3) ppm.

Diastereomer/tautomer **3b**: ^{1}H NMR (*THF*-d_8, δ, 500 MHz, 25°C): 17.55 (s, OH), 14.68 (s, 2OH), 14.10 (s, 2OH), 8.02 (d, $J = 8$ Hz, naphthyl-H-2), 7.76 (d, $J = 8$ Hz, naphthyl-H-4), 7.73 (d, $J = 8$ Hz, naphthyl-H-5), 7.67 (d, $J = 8$ Hz, naphthyl-H-8), 7.41 (m, naphthyl-H-3, H-6, H-7), 7.35 (s, 2H-ar), 6.66 (s, 2H-ar), 5.34 (m, CH), 2.79 (s, $2CH_3$), 1.72 (s, CH_3) ppm.

Diastereomers/tautomers **3a** + **3b** (1:3): ^{1}H NMR (*DMSO*-d_6, δ, 500 MHz, 25°C): 18.45 (s, OH), 14.67 (bs, 2OH), 14.07 (bs, 2OH), 8.20 (d, $J = 8$ Hz, naphthyl-H-2), 8.01 (d, $J = 8$ Hz, naphthyl-H-4), 7.97 (d, $J = 8$ Hz, naphthyl-H-5), 7.71 (d, $J = 8$ Hz, naphthyl-H-8), 7.61 (m, naphthyl-H-3, H-6, H-7), 7.46 (s, 2H-ar), 6.59 (s, 2H-ar), 5.28 (m, CH), 2.75 (s, $2CH_3$), 1.61 (s, CH_3) ppm; UV/Vis (*THF*, $c = 1.5 \cdot 10^{-3}$ mol · dm^{-3}, 25°C): $\lambda_{max}(\varepsilon) = 598$ (35821), 554 (25471), 517 (12018), 482 (17129), 384 (15062), 336 (31221) nm; CD (*THF*, $c = 1.5 \cdot 10^{-3}$ mol · dm^{-3}, 25°C): $\lambda_{max}(\Delta\varepsilon) = 598$ (−7.2), 554 (−3.8), 482 (−2), 367 (+1.9), 336 (−2.9) nm; IR (KBr): $\nu = 3427$, 1588, 1554, 1497, 1258 cm^{-1}.

Hypericin-3-(1S)-camphanate (**4**; $C_{40}H_{28}O_{11}$)

To a cold (−40°C) stirred solution of 20 mg of the Na salt of **1** (0.04 mmol) and 40 mm^3 pyridine (0.48 mmol) in 14 cm^3 *THF*, a solution of 10 mg (1*S*)-(−)-camphanic chloride (0.044 mmol) in 1 cm^3 of *THF* was added dropwise under Ar. After 1 h, the cooling bath was removed, the reaction was quenched with H_2O, and the mixture was extracted with EtOAc. The combined organic extracts were washed with brine (2x), H_2O (1x), and dried over Na_2SO_4. Filtration, concentration, and purification of the red violet residue by flash column chromatography ($CHCl_3$:MeOH = 20:1) afforded 16 mg (60%) of **4** as a 1:1.2 diastereomeric mixture. In solution at room temperature, the two diastereomers equilibrated to a final ratio of approximately 1:5.

Diastereomer **4a**: ^{1}H NMR ($CDCl_3$/*DMSO*-d_6, δ, 500 MHz, 25°C): 14.06 (s, OH), 13.95 (s, OH), 13.77 (s, OH), 13.57 (s, OH), 11.71 (s, OH), 7.31 (s, H-ar), 7.30 (s, H-ar), 7.28 (s, H-ar), 6.92 (s, H-ar), 2.73 (s, CH_3), 2.72 (s, CH_3), 2.52 (m, 1H, CH_2-camphane), 1.85 (m, 1H, CH_2-camphane), 1.71 (m, 1H, CH_2-camphane), 1.50 (m, 1H, CH_2-camphane), 1.06 (s, CH_3-camphane), 1.04 (s, CH_3-camphane), 1.02 (s, CH_3-camphane) ppm; ^{1}H NMR (pyridine-d_5, δ, 500 MHz, 25°C): 15.52 (bs, 2OH), 14.78 (bs, 2OH), 7.47 (s, 2H-ar), 7.21 (s, 2H-ar), 2.73 (s, CH_3), 2.72 (s, CH_3), 2.46 (m, 1H, CH_2-camphane), 1.95 (m, 1H, CH_2-camphane), 1.78 (m, 2H, CH_2-camphane), 1.27 (s, CH_3-camphane), 1.15 (s, CH_3-camphane), 1.06 (s, CH_3-camphane) ppm; ^{1}H NMR ($CDCl_3$/*DMSO*-d_6, δ, 500 MHz, 40°C): 14.06 (s, OH), 13.95 (s, OH), 13.77 (s, OH), 13.58 (s, OH), 11.62 (s, OH), 7.34 (s, H-ar), 7.33 (s, H-ar), 7.31 (s, H-ar), 6.94 (s, H-ar), 2.75 (s, CH_3), 2.74 (s, CH_3), 2.52 (m, H, CH_2-camphane), 1.90 (m, H, CH_2-camphane), 1.72 (m, H, CH_2-camphane), 1.50 (m, H, CH_2-camphane), 1.08 (s, CH_3-camphane), 1.06 (s, CH_3-camphane), 1.04 (s, CH_3-camphane) ppm; CD ($CHCl_3$/*DMSO*, $c = 2 \cdot 10^{-5}$ mol · dm^{-3}, 25°C): $\lambda_{max}(\Delta\varepsilon) = 594$ (+44), 550 (+25), 513 (+8), 460 (−46), 416 (−54), 358 (+22), 324 (−66) nm.

Diastereomer **4b**: ^{1}H NMR ($CDCl_3$/*DMSO*-d_6, δ, 500 MHz, 25°C): 14.05 (s, OH), 13.94 (s, OH), 13.74 (s, OH), 13.55 (s, OH), 12.03 (s, OH), 7.31 (s, H-ar), 7.30 (s, H-ar), 7.27 (s, H-ar), 6.92 (s, H-ar), 2.72 (s, CH_3), 2.71 (s, CH_3), 2.45 (m, 1H, CH_2-camphane), 1.85 (m, 1H, CH_2-camphane), 1.71 (m, 1H, CH_2-camphane), 1.50 (m, 1H, CH_2-camphane), 1.08 (s, CH_3-camphane), 1.06 (s, CH_3-camphane), 0.88 (s, CH_3-camphane) ppm; ^{1}H NMr (pyridine-d_5, δ, 500 MHz, 25°C): 15.52 (bs, 2OH), 14.78 (bs, 2OH), 7.47 (s, 2H-ar), 7.42 (s, 2H-ar), 2.79 (s, CH_3), 2.77 (s, CH_3), 2.60 (m, 1H, CH_2-camphane), 2.11 (m, 1H, CH_2-camphane), 1.87 (m, 1H, CH_2-camphane), 1.62 (m, 1H, CH_2-camphane), 1.13 (s, CH_3-camphane), 1.12 (s, CH_3-camphane), 1.11 (s, CH_3-camphane) ppm; ^{1}H NMR ($CDCl_3$/*DMSO*-d_6, δ, 500 MHz, 40°C): 14.06 (s, OH), 13.94 (s, OH), 13.75 (s, OH), 13.56 (s, OH), 11.97 (s, OH), 7.34 (s, H-ar), 7.33 (s, H-ar), 7.30 (s, H-ar), 6.95 (s, H-ar), 2.75 (s, CH_3), 2.73 (s, CH_3), 2.52 (m, H, CH_2-camphane), 1.90 (m, H, CH_2-camphane), 1.72 (m, H, CH_2-camphane), 1.50 (m, H, CH_2-camphane), 1.10 (s, CH_3-camphane), 1.08 (s, CH_3-camphane), 0.90 (s, CH_3-camphane) ppm; CD ($CDCl_3$/*DMSO*, $c = 2 \cdot 10^{-5}$ mol · dm^{-3}, 25°C): $\lambda_{max}(\Delta\varepsilon) = 594$ (−44), 550 (−25), 513 (−8), 460 (+46), 416 (+54), 358 (−22), 324 (+66) nm.

Diastereomers/tautomers **4a** + **4b**: UV/Vis ($CHCl_3$/*DMSO*, $c = 2 \cdot 10^{-5}$ mol · dm^{-3}, 25°C): $\lambda_{max}(\varepsilon) = 594$ (48366), 550 (12348), 513 (8948), 460 (27806), 416 (12372), 358 (6518), 324 (37981) nm; ^{13}C NMR (pyridine-d_5, δ, 125 MHz, 25°C): 185.3 (2CO), 179.5 (CO), 177.0 (CO), 170.8 (2 C-ar), 170.3 (2 C-ar), 163.0 (2 C-ar), 143.9 (2 C-ar), 129.0 (2 C-ar), 128.0 (2 C-ar), 123.3 (2 C-ar), 122.1 (2 C-ar), 121.5 (2 C-ar), 121.1 (2 C-ar), 11.97 (2 CH-ar), 110.3 (2 C-ar), 107.4 (2 CH-ar), 103.9 (2 C-ar), 92.8 (C-camphane), 55.6 (C-camphane), 54.4 (C-camphane), 31.7 (CH_2-camphane), 29.9 (CH_2-camphane), 17.5 (CH_3-camphane), 17.4 (CH_3-camphane), 10.4 (CH_3-camphane) ppm; Ir (KBr): $\nu = 3403$, 2925, 1793, 1762, 1625, 1602, 1474, 1262 cm^{-1}; MS (CI, CH_4, 70 eV, 200°C): m/z (%) = 685 (100; M^+), 669 (10), 505 (8), 43 (8).

Acknowledgments

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