Inter- and Intramolecular Chiral Stacking of Acridine Derivatives

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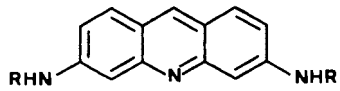
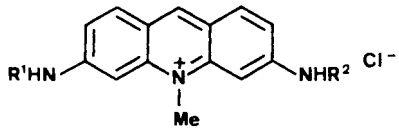
Sugar derivatives of diaminoacridine stack in aqueous solution like the parent compound. This is shown by CD measurements which give concentration-depending CD couplets. An extraordinarily strong CD couplet is obtained around 255 nm when two units of hydroxyacridine (base changed for solubility reasons) are connected by a 6-membered chain.

Stacking is best known for purine and pyrimidine bases in polynucleotides and nucleic acids[1] but does not necessarily require covalent bonding between the individual bases. It has long been known for other π systems, too, as e.g. for cyanine dyes[2], acridine derivatives[3], or indoles[4]. Stacking manifests itself by changes in the UV/VIS and fluorescence spectra[5]; if chiral molecules are involved, CD couplets can be produced[6], whose magnitudes are very sensitive to the type of chromophore and conditions of measurements. Since we had several derivatives of 3,6-diaminoacridine (proflavine) (**1a**–**1c**) and their quaternary N(10)-methyl (acriflavine) salts available (**2a**–**2c**), which were rendered chiral by N-glycosylation[7], we investigated their CD spectra in order to detect such stacking behaviour.



1a R = β-D-Glucose
1b R = β-D-Lactose
1c R = β-D-Galactose



2a $R^1 = R^2 = \beta$-D-Glucose
2b $R^1 = H$, $R^2 = \beta$-D-Lactose
2c $R^1 = R^2 = \beta$-D-Ribose

Both types of compounds show intense UV absorptions around 450 and 260 nm, and with all D-glycosides two positive CD couplets are observed in water (Table 1), whose magnitudes decrease with dilution (**1c**: Figure 1). For the diglucoside **2a** we measured the CD around 460 nm in water over a concentration range of 300:1 (Figure 2) and found that the amplitude a of the CD couplet[8] follows the equation

$$a = 34.6\{1 - \exp(-0.82[c + 0.15])\}$$

$a = \Delta\varepsilon_{max,1} - \Delta\varepsilon_{max,2}$; c in mmol/l).

In contrast, only a unisignate weak negative Cotton effect was measured in dimethyl sulfoxide solution. Obviously, all these acridine derivatives stack in water solution, and because of the chiral sugar moieties their long axes are not parallel to each other but twisted (in addition to this, translations may also appear). From the positive sign of the two CD couplets it follows that the sense of twist is as shown in Figure 3.

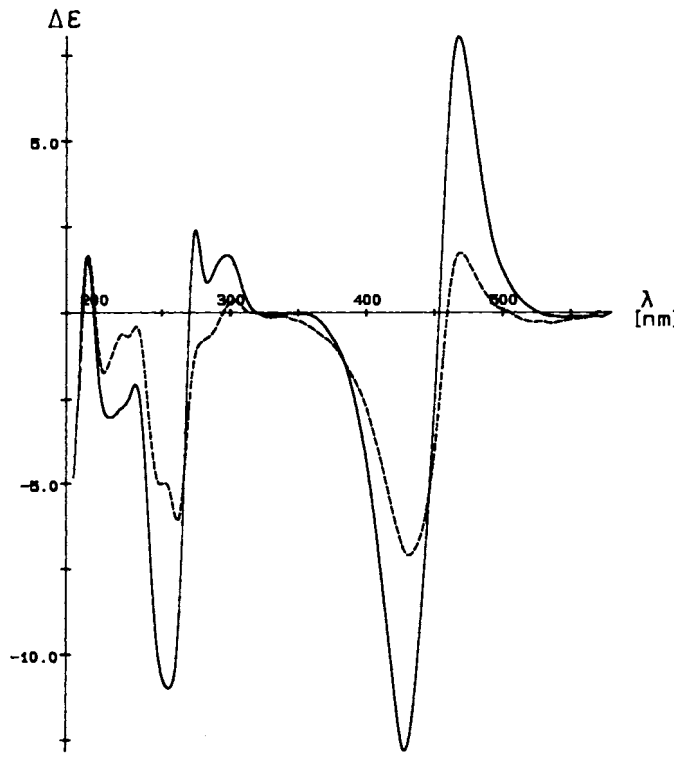


Figure 1. CD of **1c** in aqueous solution at concentrations of 0.404 (———) and 0.081 mmol · l^{-1} (-----)

Both transitions, that leading to the short-axis polarized 450-nm band as well as the one giving the long-axis polarized 260-nm band, should have the same sign, since the cross-interaction between these two transition moments is small because of their great difference in energies. It is, however, noteworthy, that the CD couplet within the

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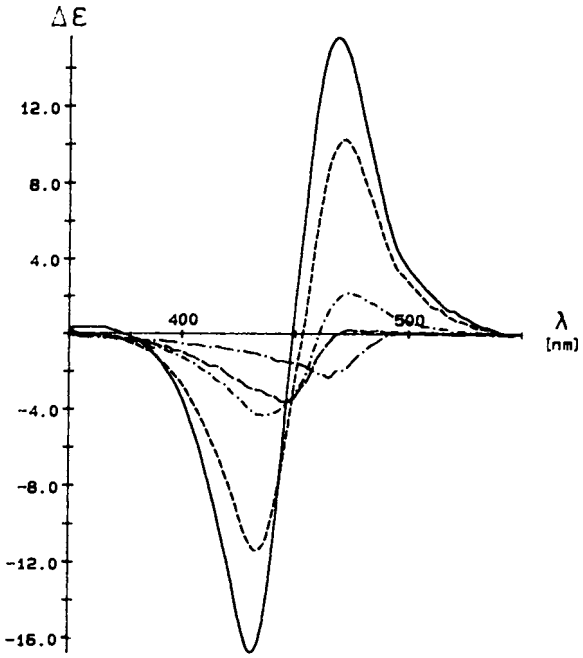
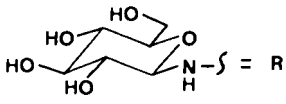
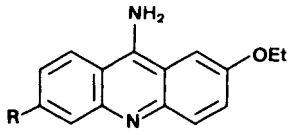


Figure 2. CD around 450 nm of **2a** in DMSO (1.8273 mmol · l^{-1} (— · — ·) and in water at different concentrations: 3.1924 (———), 1.0816 (-----), 0.1132 (— · — ·), and 0.0108 mmol · l^{-1} (— — —)

stronger 260-nm absorption band is smaller than that around 450 nm.

Compound **3** shows a similar behaviour, with the amplitude of the positive CD couplet around 270 nm of similar magnitude as for the diglycosides, whereas the second (also positive) couplet around 370 nm is quite small. Both decrease again after dilution. It is furthermore interesting to note that no such CD couplet could be observed for the diglucoside of ethidium bromide (**4**), although the free diamine is known to intercalate similarly into nucleic acids like proflavine and acriflavine.





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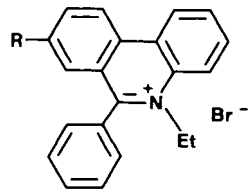

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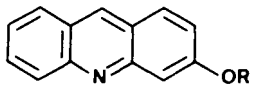
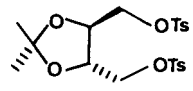


5a R = H
5b R = Me



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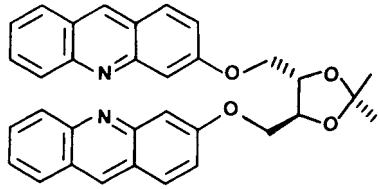

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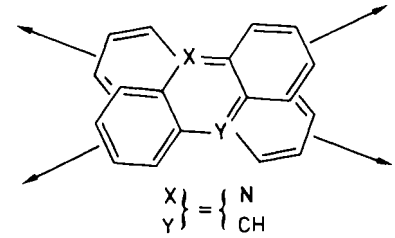

Figure 3. Approximate geometrical arrangement of the two acridine moieties of **2a** necessary for a strong positive CD couplet

Table 1. CD spectral data

Compound	Solvent[a]	Concentration [mmole ℓ^{-1}]	Δε(λ[nm])
1a	W	0.6153	+2.98(464), -3.19(403), +0.6(330sh), -8.1(260)
1b	W	0.3473	+4.55(467), -9.86(428), +0.78(330), +1.29(305), -10.4(264), -6.4(249)
1c	W	0.4040	+8.05(468), -12.79(428), +1.68(298), +2.39(275), -10.98(256), -3.0(212), +1.6(195)
		0.081	+1.75(469), -7.10(431), +0.35(303), -6.08(261), -5.0(249), -1.7(207)
2a	W	1.0816	+10.81(469), -11.31(431), +1.40(295), +5.0(273), -10.0(256)
		0.1132	+1.98(472), -3.64(436), +0.31(308), -2.7(280), -4.5(262)
		0.0108[b]	+0.23(474), -3.62(445)
		3.1924[b]	+15.62(468), -16.76(431)
	DM	1.8273	+0.07(504), -2.33(465)
2b	W	2.5402	+8.70(471), -8.75(432), +0.39(310), -9.78(251)
2c	W	0.4040	+7.84(472), -5.03(434), +0.37(370), +1.68(291), +4.08(273), -2.84(256), -3.20(247), -1.7(226)
		0.082	+2.0(467), -0.5(350), +0.4(285), +1.4(≈276), -0.8(≈250)
3	W	0.509	+0.07(455), -0.14(426), -0.85(412), +1.66(379), -1.43(349), +3.1sh(295), +7.4(279), -12.4(259), -3.1(206)
		0.103	-0.53(≈425), -0.63(408), +0.49(381), -1.08(351), +1.1(295), +2.9(280), -6.9(263), -2(206)
4	W	0.322	-0.36(480), +0.02(376), +0.20(342), -0.5(318), -4.5(286), -2.0(233), -3.8(212)
7	AN	1.100	+0.03(416), -0.5sh(391), -1.16(368), -1.31(359), -0.91(339), -0.8sh(330), -0.5sh(314), -181.3(256), +102.0(245)
		0.314	-0.81(388), -1.55(371), -1.83(357), -161(257), +83(249), +83(246)
		0.112[b]	-267(256), +121(248), +117(245)
		0.063[b]	-242(256), +105(249), +105(245)
		0.026[b]	-252(256), +109(248), +111(245)
	ET	0.710	-0.25sh(402), -0.33(390), -1.10(349), -0.62(332), -0.38(315), -61.3(256), +20.8(250), +20.9(246)
		0.073[b]	-63(257), +21(250), +20(246)
		0.025[b]	-58(257), +20(250), +20(246)
	DN	1.158[c]	-0.5sh(390), -1.37(379), -1.73(256), -0.9sh(330)
		0.347[b]	-237(258), +101(251), +101(248)
		0.070[b]	-240(258), +95(250), +95(247)
		0.013[b]	-223(259), +90(251), +90(248)
	HF	0.911	-0.07(443), +0.23(425), +0.28(410), -10.5sh(265), -42.2(258), +20.0(250)
		0.209	-6.23(365), -10sh(267), -42.1(259), +18.7(250)
		0.045	-6.3(365), -11sh(267), -38(258), +14(250)

[a] Solvents: AN = acetonitrile, DM = DMSO, DN = dioxane, ET = ethanol, HF = hexafluoroacetone hydrate, W = water. — [b] CD couplet only. — [c] Couplet not measured.

If two molecules which are prone to stack are connected by a bridge of three to five atoms then they will do so also in this "dimer"[4], but the two units can of course not dissociate by dilution. If such a bridge is made chiral, then one could again expect the appearance of CD couplets, and so we tried to synthesize such "dimers" of acriflavine or proflavine. The known tendency for aggregation did, however, not allow to purify such derivatives; we thus turned to 3-hydroxyacridine (**5a**)[9] as starting material. As chiral bridge we used the easily available dimethyl acetal **6**, prepared from (+)-tartaric acid according to the literature[10]. **6** (but not its corresponding diiodide) reacted smoothly with **5a** to afford **7**. For comparison the methyl ether **5b** was synthesized by methylation of **5a** with dimethyl sulfate.

Both **5a** and **7** show a relatively strong UV absorption with pronounced fine structure between 300 and 400 nm, and a very strong band about 255 nm. That this first band is actually a superposition of two is seen much better in the CD spectrum of **7** in ethanol solution: one negative Cotton effect has its maximum around 390 nm, the other, also negative one, around 350 nm. Both show fine structure but no indication of couplet character, and they may be associated with the *p*- and α-band of anthracene or acridine, which appear nearly at the same positions (the *p*-bands have been assigned to the absorptions at 380 nm for anthracene and at 347 nm for acridine, the α-band of anthracene to the one at 355 nm[11]).

On the other hand within the β-band around 255 nm a huge negative CD couplet is observed with an amplitude up to −350 (acetonitrile, dioxane), which is conservative although the positive branch is only half as large as the negative one, but its halfband-width is larger. As expected, in ethanol the amplitude of this couplet drops to about −80, since ethanol can solvate the acridine moiety much better and is thus partially breaking the stacking (Figure 4).

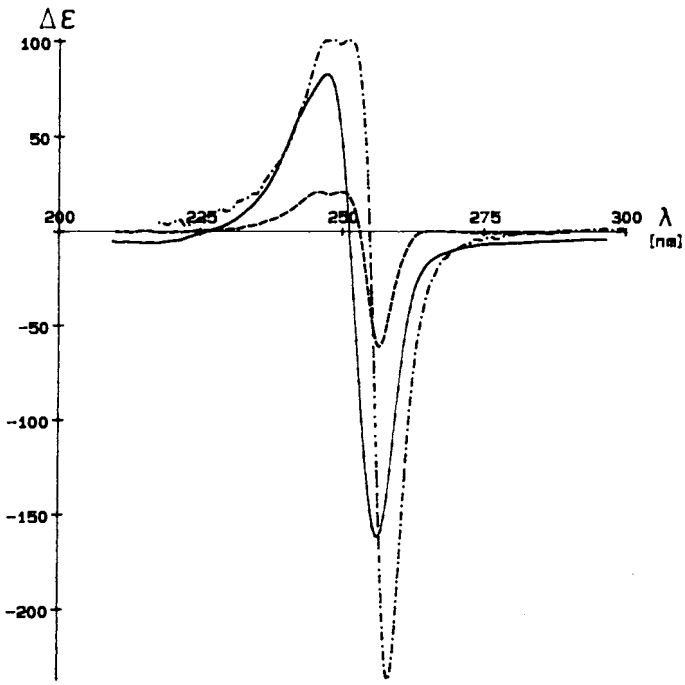

Figure 4. CD couplet around 255 nm of **7** in acetonitrile (———), dioxane (— · — ·), and ethanol (-----) solution

It is hence only the interaction of the long-axis polarized transition moments, which is chiral, whereas the two short-axis polarized transition moments should be either nearly parallel or perpendicular to each other. To accomodate such a conformation the two planes of the acridine moieties cannot be parallel but have to be tilted, too. The twist angle between the two units of **7** must be opposite to that given in Figure 3 for the intermolecularly stacked acridines. That indeed exciton interaction is involved is also supported by the fact that the halfband-width of this strong β-band of **7** is larger than that of the ether **5b**, with concomitant hypochromism. Although such exciton interaction can be quite far-reaching it cannot come from an "elongated" nonstacked conformation, because for that distance the amplitude of the couplet should be much smaller.

CD is thus also a very good method to detect stacking in solution for such chiral mono- and dimeric acridine derivatives, and to get information about the conformation leading to such aggregation.

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Experimental

NMR spectra were recorded on a Bruker AM-400 in $CDCl_3$ solution, IR spectra on a Perkin-Elmer 1310 ($CHCl_3$), and mass spectra on MAT CH-5 or CH-7 spectrometers. The CD was measured with an ISA-Jobin-Yvon dichrographe Mark III.

3-Hydroxyacridine (**5a**): A suspension of 1.6 g of finely ground 3-aminoacridine[12] in an ice-cold mixture of 8.8 g of water and 7 g of concd. H_2SO_4 is sealed under argon in a thick-walled glass tube and kept for 8 h at 195°C. After cooling, the resulting suspension is filtered, the brown crystals are washed twice with a few millilitres of water and dissolved in 20 ml of 1 N NaOH. After filtration the solution is weakly acidified with 25% acetic acid, and the collected precipitate is dried at 140°C in vacuo over P_2O_5. This product (m.p. 270.5−274°C, 53% yield, ref.[9] 292°C) was pure enough for the preparation of its methyl ether **5b**.

3-Methoxyacridine (**5b**): To an aqueous solution (5 ml) of 31 mg (0.77 mmol) of NaOH and 150 mg (0.77 mmol) of 3-hydroxyacridine (**5a**) is added under stirring 97 mg (0.77 mmol) of dimethyl sulfate and the solution is kept at room temperature over night. The dark precipitate is washed with a few millilitres of dilute NaOH, dissolved in CH_2Cl_2, dried over sodium sulfate, and chromatographed twice on silica gel (CH_2Cl_2/ethanol/triethylamine, 70:1:0.7; petroleum ether/acetone/triethylamine, 100:10:1) under medium pressure. The obtained yellow dye (65 mg, 40% yield) was then sublimed at 110°C in vacuo; m.p. 85°C (ref.[13] 89−90°C). − ^{1}H NMR: δ = 3.96 (s, 3H, CH_3), 7.15 (dd, 1H, 2-H), 7.40−7.44 (m, 2H, 4-H, 7-H), 7.69−7.74 (m, 1H, 6-H), 7.78 (d, 1H, 1-H), 7.88 (d, 1H, 8-H), 8.12 (dd, 1H, 5-H), 8.55 (s, 1H, 9-H). − ^{13}C NMR: δ = 55.5 (CH_3), 105.0 (C-4), 121.1 (C-2), 122.8 (C-9a), 124.6 (C-7), 125.4 (C-8a), 128.2 (C-8), 128.6 (C-5), 129.3 (C-1), 130.3 (C-6), 135.7 (C-9), 149.0 (C-4a), 150.7 (C-10a), 161.4 (C-3). − IR ($CHCl_3$): $\tilde{\nu}$ = 2950 (double band, aliphatic C−H), 1635, 1620 (C=N), 1280, 1170 cm^{-1} (C−O). − MS: *m/z* (%) = 209 (100) [$M^{+\cdot}$], 179 (14) [$M^+ - CH_2O$], 166 (62) [$M^+ - CH_3 - CO$]; relative molecular mass: calcd. 209.0841, found 209.0841.

$C_{14}H_{11}NO$ (209.08) Calcd. N 6.70 Found N 6.64

(4S,5S)-trans-4,5-Bis(3-acridinyloxymethyl)-2,2-dimethyl-1,3-dioxolane (**7**): A suspension of 300 mg (1.5 mmol) of finely powdered **5a** and a large excess of potassium carbonate in 7 ml of DMF is heated to 80°C, then 360 mg (0.77 mmol) of (4*S*,5*S*)-(−)-*trans*-4,6-bis(tosyloxymethyl)-2,2-dimethyl-1,3-dioxolane (**6**)[10] is added. After heating for several hours to 110°C, the solution is cooled, filtered,

and washed several times with CH_2Cl_2. The residue from the evaporated combined organic phases is chromatographed on silica gel (20–45 μm, 3 bar) with CH_2Cl_2/ethanol/triethylamine, 100:2:1) and gives 36 mg (13% yield) of a yellow solid of m.p. 222–223°C. – ^{1}H NMR: δ = 1.58 (s, 6H, 2 CH_3), 4.43 (m, 4H, 2 CH_2), 4.56 (m, 2H, 4-, 5-H), 7.26 (dd, 2H, 2′-, 2″-H), 7.49 (m, 4H, 4′-, 4″-, 7-, 7″-H), 7.76 (m, 2H, 6′-, 6″-H), 7.84 (d, 2H, 1′-, 1″-H), 7.94 (d, 2H, 8′-, 8″-H), 8.18 (d, 2H, 5′-, 5″-H), 8.64 (s, 2H, 9′-, 9″-H). – IR: $\tilde{\nu}$ = 2998, 2970 (aliphat. C–H), 1635, 1620 (C=O), 1380 (double band, gem. CH_3), 1280 and 1130 cm^{-1} (C–O). – MS: *m/z* (%) = 516 (47) [$M^{\cdot+}$], 501 (5) [$M^+ - CH_3$], 322 (97) [$M^+ - C_{13}H_8NO$], 195 (100) [$C_{13}H_9NO^+$], 44 (95) [$C_2H_6N^+$], 43 (98) [$C_2H_5N^+$]; relative molecular mass: calcd. 516.2049, found 516.2052.

$C_{29}H_{28}N_2O_4$ (516.21) Calcd. N 5.43 Found N 4.94

CAS Registry Numbers

1a: 84207-91-0 / **1b**: 84207-97-6 / **1c**: 84207-93-2 / **2a**: 84207-99-8 / **2b**: 84208-04-8 / **2c**: 119619-83-9 / **3**: 119619-84-0 / **4**: 119619-85-1 / **5a**: 7132-70-9 / **5b**: 23043-46-1 / **6**: 37002-45-2 / **6** (diiodide derivative): 58342-57-7 / **7**: 119619-86-2 / 3-aminoacridine: 581-29-3 / 3-aminoacridine sulfate: 119619-87-3

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