

## Controlling the Helicity of Tubular J-Aggregates by Chiral Alcohols

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The achiral 3,3'-bis(3-carboxypropyl)-5,5',6,6'-tetrachloro-1,1'-dioctylbenzimidacarbocyanine dye ( $C_8O_3$ ) self-aggregates in aqueous solution to form optically active helical J-aggregates. A three-phasic circular dichroism (CD) spectrum with positive Cotton effect at 568 nm, negative Cotton effect at 582 nm, and positive Cotton effect at 605 nm (+,−,+) is usually found and ascribed to molecular excitons. The predominance of the (+,−,+) signature means that the generation of chirality is enantioselective. The CD signal increases by a factor of about 100 when the chiral (*R*)-2-octanol is added to an aggregated solution, and the CD signal is further amplified by a factor of about 5 when the alcohol is present in the solvent prior to aggregation. The signature of the CD signal reverses if the enantiomeric (*S*)-2-octanol is used instead, and for a molar alcohol/dye ratio *R* of about 20, the spectra become mirror images in good approximation; i.e., the chirality of the alcohol and the CD spectra of aggregates are correlated. The net effect of chiral alcohols is an increased excess of aggregates with a particular handedness due to induced circular dichroism. Samples containing (*S*)-2-octanol showed that their "reversed" CD spectra (−,+,−) signature are not fully stable when stored for longer times at ambient temperature. The spectral changes indicate a gradually increasing contribution from aggregates with the opposite sense of handedness ((+,−,+) signature). Electron micrographs reveal left helix handedness for the majority of aggregates in the presence of (*R*)-2-octanol ((+,−,+) signature), while right helix handedness predominates for the aggregates in the presence of (*S*)-2-octanol ((−,+,−) signature).

### Introduction

The formation of supramolecular architectures by self-assembly to obtain a well-defined microscopic organization and macroscopic characteristics is one main objective of supramolecular chemistry.<sup>1–3</sup> Controlling helicity,<sup>1,4,5</sup> in particular, has attracted strong interest because of the great opportunities for applications in material science, chemical sensing, and enantioselective catalysis. The spontaneous self-assembly of molecules into complex structures is the result of a multitude of effective noncovalent interaction processes such as hydrogen bonding, electrostatic, dipole–dipole, van der Waals, and solvophobic interactions. A variety of artificial self-assembled chiral structures comprising helical fibers and twisted ribbons of sugars,<sup>6</sup> phospholipids<sup>7</sup> and gemini surfactants,<sup>8</sup> helicenes,<sup>4</sup> helical metal complexes,<sup>1</sup> block copolymers,<sup>9</sup> or the coiled aggregates of (crown ether phthalocyaninato) polysiloxanes<sup>10</sup> have been created from chiral or racemic mixtures employing the spectrum of noncovalent interactions in their design. Despite the efforts and the success in design and synthesis, the detailed mechanism of chirality transfer from the chiral molecular building block to the chiral superstructure is often unknown.

However, it is still more surprising that chiral supramolecular structures can also be obtained by self-assembly from achiral molecular building units.<sup>11,12</sup> Recently, the formation of macroscopic chiral domains by disklike (benzenehexamine derivatives)<sup>13</sup> and banana-shaped molecules (benzylideneaniline com-

pounds)<sup>14</sup> in liquid crystals and the spontaneous resolution of enantiomers in two-dimensional monolayers<sup>5,15</sup> have been reported. Beyond that, it has been demonstrated that different achiral porphyrine dyes<sup>16,17</sup> form chiral molecular aggregates in aqueous solutions by self-assembly.

Recently, it has been shown by Dähne and co-workers<sup>18–21</sup> that certain derivatives of the planar 5,5',6,6'-tetrachlorobenzimidacarbocyanine chromophore with hydrophobic alkyl and hydrophilic acidic substituents also form optically active molecular aggregates in aqueous solution. These aggregates show the typical spectroscopic features of J-aggregates, which are characterized by the appearance of at least one new and red-shifted optical absorption band (J-band) in combination with resonance fluorescence.<sup>22–24</sup>

The optical properties of the J-aggregates of cyanine dyes are determined by the electronic coupling between the transition dipoles, which are extremely large because of the high polarizability of the  $\pi$ -electrons of the polymethine backbone. In addition, the sizes of these dipoles lead to strong intermolecular excitation transfer interactions that enable efficient energy migration over many molecules,<sup>25</sup> and thus, these aggregates are favored as model systems for mimicking photosynthetic light-harvesting complexes.<sup>26,27</sup> The strong polarizability causes dispersion forces between neighboring molecules, which are the major driving forces for aggregation of these polymethine dyes.<sup>28–30</sup> Owing to their dominance in self-organization, other noncovalent interactions play only a secondary role in effecting the particular structures of aggregates of most of the cyanine dyes investigated hitherto. This could be changed when the peculiar properties of the polymethine dyes were combined with structural elements typical of amphiphilic molecules by intro-

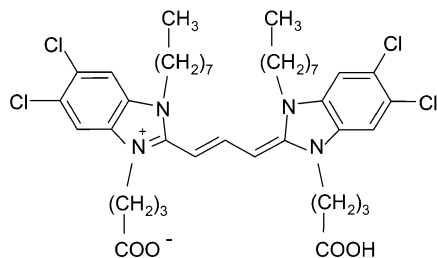
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ducing hydrophobic side chains on one side and hydrophilic side groups on the other side of the chromophore.<sup>19,31</sup> Solvophobic interactions then gain in significance. Owing to the combination of these outstanding features, this novel class of cyanine dyes has been termed<sup>32</sup> *amphipipes* (*amphiphiles* with pigment interaction performing energy-migration). With use of cryotransmission electron microscopy (cryo-TEM), it was discovered that these dyes aggregate in a variety of complex supramolecular structures that had not been described for J-aggregates until then.<sup>33</sup>

The most prominent derivative in this class of substituted chromophores is the 3,3'-bis(3-carboxypropyl)-5,5',6,6'-tetrachloro-1,1'-dioctylbenzimidacarbocyanine dye (abbreviated as C<sub>8</sub>O<sub>3</sub>)



which forms optically active J-aggregates in aqueous solutions by self-assembly.<sup>18,19</sup> Cryo-TEM revealed<sup>33</sup> a superhelical architecture of the aggregates. The formation of ropelike assemblies, built up by several tubular strands of ~10 nm cross-sectional diameter and ~4 nm wall thickness, correlates with the observed circular dichroism. Because of the planarity and hence achirality of the C<sub>8</sub>O<sub>3</sub> molecule in solution, we were obliged to assume that the circular dichroism arises from a spontaneous chiral symmetry-breaking in the arrangement of adjacent molecules within the single strands.

The formation of bilayer structures by self-assembly can be explained by the amphiphilic nature of the dye molecule, in which the hydrophilic acido groups are exposed to water, whereas the hydrophobic tails are shielded from water. But instead of forming flat bilayers or large spherical vesicles with minimum curvature (as one would expect for the lowest energy state of the bilayer),<sup>34</sup> high-curvature tubular structures are formed. Similar tubular structures are often found with chiral molecules,<sup>6,35–37</sup> where the tubules occasionally reveal striations in a screwlike manner, indicating that they are actually organized by tightly twisted helical ribbons.<sup>6,38</sup> One could thus speculate that the elementary tubular strands forming the ropelike C<sub>8</sub>O<sub>3</sub> J-aggregates are also built up by helical ribbons; however, the decisive experimental proof is missing until now.

For any chiral assembly, it is challenging to find a method to control the enantioselectivity upon structure formation. Several successful experiments have been described in the literature in which the supramolecular helicity has been induced or controlled by chiral auxiliaries,<sup>8,39–41</sup> changing the intermolecular interactions between the molecules (i.e., by pH variation or metal ion complexation),<sup>10,42</sup> or by external stimuli (i.e., by stirring or temperature treatment).<sup>43–46</sup> In particular, the approach of producing helical J-aggregates by a templating technique, in which  $\alpha$ -helical peptides or double-helical DNA<sup>47–50</sup> molecules serve as templates for controlling the structure of the resulting aggregates, has been successfully proven.

Though the C<sub>8</sub>O<sub>3</sub> J-aggregate generates chirality by itself, it would be interesting if the expression of chirality could be controlled. The circular dichroism (CD) spectrum of the pure aggregate is characterized by three subbands with alternating

signs (two maxima (+) and a minimum (–) between).<sup>33</sup> This type of CD spectrum, in particular a (+,–,+) subband pattern, was measured in about 98% of all freshly prepared solutions.<sup>51</sup> We concluded that nearly all samples contain an excess of aggregates of the same handedness, while its absolute sense remained unknown. Electron micrographs revealed a helical superstructure of the J-aggregates, exhibiting both right- and left-handed twist (the pitch is on the order of several hundreds of nanometers but generally varies), whereas the amount of left-handed helices dominates.<sup>33</sup>

Reversing the sense of handedness by addition of large amounts of enantiomerically pure tartrate ions was tried,<sup>51</sup> but reproducing the previously reported reversal<sup>18</sup> with the present dye samples failed. This finding is probably related to the fact that in the previous study the dye samples contained a significant amount of the dye precursor<sup>31</sup> that could have modified the molecular packing as well as the aggregates' supramolecular structure.

Quite recently we observed, however, that upon addition of achiral short- and long-chain alcohols, the molecular packing can be modified in a manner that strongly enhances the CD signal.<sup>52</sup> Inspired by this finding and using the same strategy for the present study, we added small amounts of chiral alcohols, such as (*R*)-2-octanol, (*S*)-2-octanol, and (*S*)-2-decanol, to control the sense of handedness of the J-aggregates. The detailed investigations showed that the sense of the handedness can indeed be controlled and even reversed by the addition of the respective alcohols. The corresponding tubule and superhelix handedness were characterized by means of circular dichroism spectroscopy and electron microscopy, respectively.

## Experimental Section

**Materials and Sample Preparation.** The dye C<sub>8</sub>O<sub>3</sub> was supplied by FEW Chemicals (Wolfen, Germany). This dye is a mixture of about 50% dye bromide containing two carboxy groups and of about 50% of the dye betaine, which contains one carboxylic acid group and one carboxylate group as indicated in the formula. Recrystallization from dimethyl sulfoxide (DMSO) removed unreacted portions of the dye precursor (5,6-dichloro-1-octyl-2-methyl-3-(3-carboxypropyl)-benzimidazolium bromide) and in addition removed the bromide, giving the pure betaine (according to elemental analysis) with a yield of about 50%. Because of the recrystallization, the melting point increased from 170 to 203 °C. Combined HPLC/MS measurements confirmed the elemental composition of the dye betaine and its high purity (at least 98%).<sup>32,51</sup> The absorption maximum and the molar extinction coefficient in DMSO at room temperature have been found to be  $\lambda_{\text{max}} = 528$  nm and  $\epsilon_{\text{max}} = 185\,000$  ( $\pm 5000$ ) L/(mol·cm) after recrystallization. Single crystals could be grown from a 100 °C solution in DMSO by slowly cooling to room temperature in a Dewar vessel and storing it for several days in a refrigerator. The molecular mass was taken to be 849 g/mol, considering the result of X-ray structure analysis<sup>20</sup> that showed that the single crystals obtained by crystallization from DMSO contain 1/2 mol of DMSO.

For all investigations described in this paper, only dye material that was recrystallized at least one time was used, if not stated otherwise. The J-aggregates were prepared in two different ways. In the "direct route" the dye was dissolved in 10<sup>–2</sup> M NaOH as solvent through stirring at room temperature for at least 24 h. Homogeneous 2.8  $\times$  10<sup>–4</sup> M dye (stock) solutions with maximum formation of J-aggregates were thus obtained and later diluted by 10<sup>–2</sup> M NaOH for spectroscopy. Within the experimental limits of error (the maximum absor-

bance could be reproduced with an error of less than  $\pm 10\%$ ), the spectra from different stock solutions were reproducible. The magnitude of the CD signal showed generally larger scatter, but it should be emphasized that modified preparation conditions of solutions had no effect on its signature. In particular, an effect of the stirring direction during sample preparation could not be observed.<sup>51</sup> A chiral symmetry breaking caused by the stirring direction alone, as reported recently for aggregates of achiral porphyrins,<sup>43</sup> should therefore be excluded for the present dye. Additional proof comes from the second method of preparation that is called "alcoholic route".<sup>51</sup> In this case, the dye was first completely dissolved (indicated by the monomer spectrum) in methanol and then adjusted to the desired concentration to the polar solvent, which was  $10^{-2}$  M NaOH or  $10^{-2}$  M NaOH containing the long-chained chiral alcohol, for aggregation. These mixtures were first vigorously shaken by hand to become homogeneous and then stored without stirring in the dark for 24 h.

Methanol and ethanol (p.a.) were purchased from Merck, (*R*)-2-octanol and (*S*)-2-octanol (total amount of enantiomers: 99%) were purchased from Fluka, and (*S*)-2-decanol (purity 98%) was purchased from Sigma-Aldrich. Because of their low water solubility, the chiral alcohols were first dissolved in ethanol ( $180 \times 10^{-3}$  M) and then added to the aggregated dye solutions (titration experiments) or to the solvent used for the "alcoholic route" of preparation by using a microliter syringe (Hamilton). All spectroscopic measurements were carried out at  $21 \pm 1$  °C after the stock solutions had been stored for at least 1 day. Doubly distilled deionized water was used for preparing the solutions.

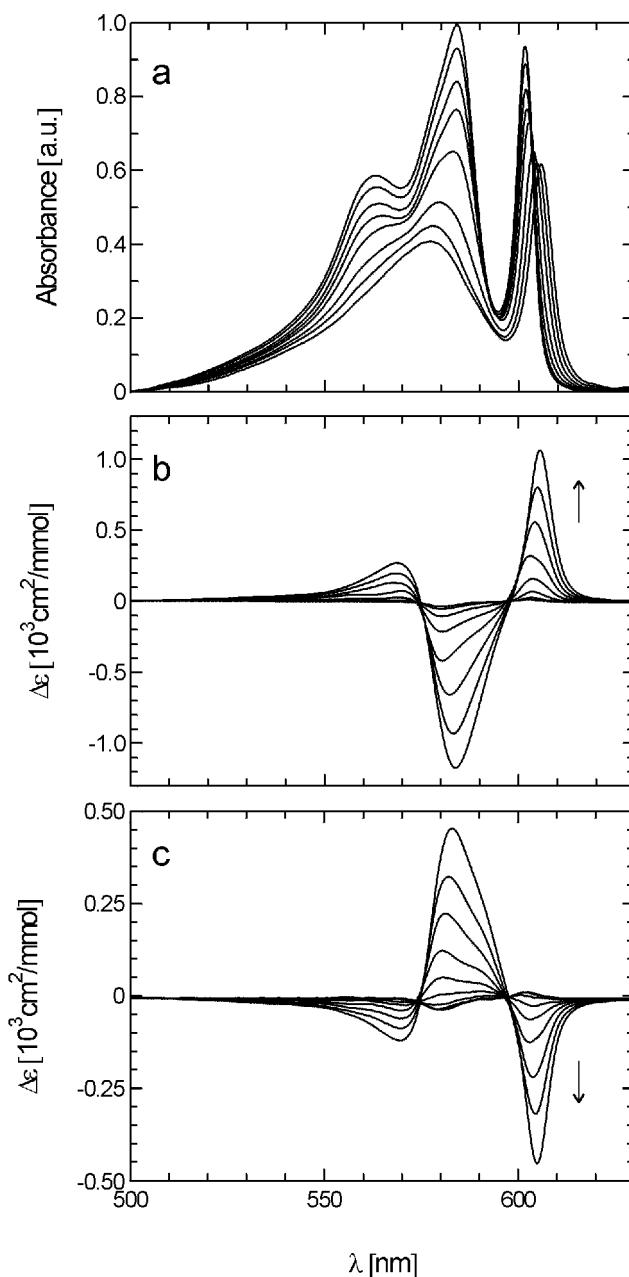
**Methods.** The absorption spectra were obtained with a Lambda 9 spectrophotometer (Perkin-Elmer), and the circular dichroism spectra were obtained with a J-715 spectropolarimeter from Jasco Corporation. Quartz cuvettes with optical path length of 0.1 cm were used.

The samples for cryo-TEM were prepared at room temperature by placing droplets (5  $\mu$ L) of the solution on hydrophilized perforated carbon-film grids (60 s of plasma treatment at 8 W using a BALTEC MED 020 device). The excess fluid was blotted off to create an ultrathin layer (typical thickness of 100 nm) of the solution spanning the holes of the carbon film. The grids were immediately vitrified in liquid ethane at its freezing point ( $-184$  °C) using a standard plunging device. The vitrified samples were transferred under liquid nitrogen into a Philips CM12 transmission electron microscope using a Gatan cryo-holder and cryostage (model 626). Microscopy was carried out at  $-175$  °C sample temperature using the microscope's low-dose protocol at a primary magnification of 58300 $\times$ . The defocus was chosen to be 0.9  $\mu$ m, corresponding to the first zero of the phase contrast transfer function at 1.8 nm.

Electron micrographs revealing the topographic information of the aggregates were taken from samples deposited on carbon films and subsequently Pt/C shadowed at an inclination angle of ca. 30° (primary magnification of 15000 $\times$ ).

## Results and Discussion

**Spectroscopy.** The absorption spectra shown in Figure 1a reveal the changes occurring upon titration of a  $6.4 \times 10^{-5}$  M aggregated dye solution with (*R*)-2-octanol. The elapsed time between each titration step was about 5 min. The top spectrum represents the absorbance of the pure dye solution. The spectrum is red-shifted compared to the absorption maximum of the dye monomer (520 nm) and exhibits the typical maxima<sup>33</sup> at 561, 582, and 600 nm. The spectrum is due to the band structure of delocalized excitons within the dye aggregate.



**Figure 1.** (a) Change of absorption spectra of a  $6.4 \times 10^{-5}$  M aqueous  $C_8O_3$  solution (containing  $10^{-2}$  M NaOH) as a function of added (*R*)-2-octanol. Molar octanol/dye ratios from top to bottom are as follows:  $R = 0, 8.5, 17.2, 25.3, 33.8, 42.0, 50.0, 58.4$ . (*R*)-2-Octanol was dissolved in EtOH and added by using a Hamilton syringe. Total EtOH concentration was 2.0 wt %. (b) Corresponding set of circular dichroism spectra. (c) Set of circular dichroism spectra after titration of a  $6.3 \times 10^{-5}$  M aqueous  $C_8O_3$  solution (containing  $10^{-2}$  M NaOH) by (*S*)-2-octanol.  $R = 0, 2.8, 5.7, 8.6, 11.4, 14.3, 17.1, 19.8, 24.1$ . Total EtOH concentration was 1.6 wt %. The arrows indicate increasing octanol concentrations.

The principal features of the excitonic spectrum of cylindrical structures have been evaluated for a model system in which the chromophores are packed to form a cylindrical aggregate.<sup>53,54</sup> For this model, a 2-fold split aggregate band is predicted with the low-energy transition oriented parallel to the long axis of the cylinder and a transition at higher energy but still red-shifted compared to the monomer that is oriented perpendicular to the long axis. For the bilayer-walled tubular J-aggregates under study here, the observed 3-fold split spectrum could be explained under the assumption that the inner and outer chromophore



layers have different absorption energies.<sup>32</sup> However, such a model cannot explain easily the transition from a 2-fold to a 3-fold split spectrum. We therefore would favor a model where the splitting is due to the molecular packing, such as for example a herringbone-like arrangement. A detailed theoretical model does not exist at present.

Upon titration of  $C_8O_3$  with (*R*)-2-octanol, the 3-fold split spectrum progressively transforms into a 2-fold split spectrum accompanied by a decrease of the total absorbance. The maximum at the longest wavelength component (around 600 nm) shifts to even longer wavelength, whereas the peaks at 561 and 582 nm are replaced by a single band with a maximum at 578 nm. This trend continues at least up to molar (*R*)-2-octanol/dye mixing ratios of  $R \approx 150$  (not shown). Previous measurements have shown<sup>52</sup> that similar changes of absorption spectra can be induced by the addition of achiral alcohols such as methanol, 1-octanol, 1-decanol, and ethylene glycol. An alcohol-modified molecular packing within the tubular elementary strands has been discussed to explain the altered spectra. It has been concluded that the long-chained alkanols are incorporated into the tubules by hydrophobic forces. Cryo-TEM showed that despite insignificant swelling by the alcohols, the tubular morphology of the elementary strands remained nearly unaltered.

At present, there is no satisfactory theoretical model for tubule formation.<sup>34</sup> We argued in an earlier study<sup>20</sup> that a fraction of the dye molecules in the  $C_8O_3$  J-aggregate could be in a twisted state serving as chiral building unit for the growth of helical aggregates. This suggestion was based on X-ray structural data that showed that the single crystals of the dye are composed of two slightly different conformers, i.e., a strictly planar one and a conformer that is twisted along the trimethine backbone by an angle of approximately  $18^\circ$ . However, since the chirality of the twisted conformer occurs in a strictly racemic ratio, one would expect the growth of J-aggregates with an equal ratio of either senses of handedness and a zero net-CD effect, provided that chiral symmetry breaking caused by stirring the solutions<sup>43,55</sup> can be excluded. In looking for a better explanation, recent experiments on tubules of phospholipids could be of importance,<sup>56–58</sup> which showed that with a pure enantiomer the probabilities of forming left- or right-handed helical ribbons in the initial process of aggregation were roughly equal, suggesting that the initial helical ribbon formation process is not strongly influenced by intrinsic molecular chirality. The authors concluded that the helical bilayer twist does not have its origin in molecular chirality but rather in a spontaneous chiral symmetry breaking.

As shown theoretically,<sup>59</sup> it is possible that tubules might be formed out of membranes that undergo a chiral symmetry-breaking transition in which they spontaneously break reflection symmetry and select one handedness, even if they are composed of achiral molecules. The authors assumed in their model that each monolayer of the bilayer membrane is characterized by its own direction of molecular tilt. If these tilt directions are only weakly coupled to each other, molecules might pack with a nonzero relative angle. In this case packing spontaneously breaks reflection symmetry and introduces a chiral order parameter, characterizing the magnitude and sign of the symmetry breaking. It can drive an instability of the flat phase, which favors curvature of the bilayer to form a tubule, just as in a system of chiral molecules. But because of statistical fluctuations, both senses of handedness should also be realized in that case in equal proportion.

The CD spectra in Figure 1b reveal strongly increased signals due to the addition of (*R*)-2-octanol. In the absence of alcohol,

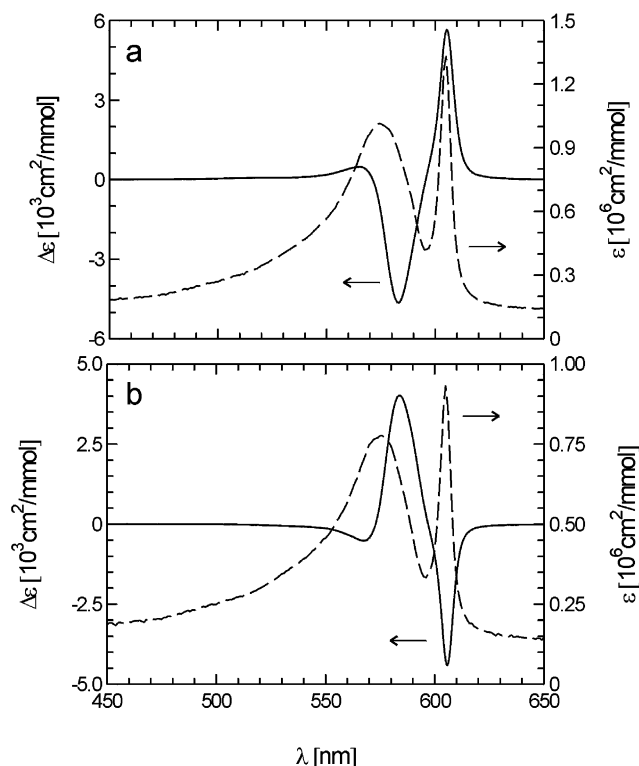
a three-phasic spectrum with positive Cotton effect at 568 nm, negative Cotton effect at 582 nm, and positive Cotton effect at 605 nm is generally found in freshly prepared solutions<sup>51</sup> and ascribed to the molecular excitons. We denote such a signature of the spectrum as (+,−,+). Up to saturation at  $R \approx 60$ , the intensity of the CD signal increases upon titration while its shape remains nearly unaltered. Only a slight red-shift of the longest wavelength subbands occurs, but the (+,−,+) subband signature, in particular, does not change. It is instructive to compare the magnitude of the alcohol-induced effects. Thus, the molar circular dichroism,  $\Delta\epsilon$ , measured in alcohol-free solution (the typical value is on the order of  $10 \text{ cm}^2/\text{mmol}$ ), is increased by a factor of about 20 when achiral alcohols are added<sup>52</sup> and by a factor of about 100 in the case of (*R*)-2-octanol.

In a very recent work,<sup>60</sup> the CD spectra for a helical cylindrical arrangement of molecules have been theoretically described in order to explain experimental results measured on rodlike chlorosomal antennae from green bacteria.<sup>61</sup> Spectra were obtained that are qualitatively similar to the one presented here, but the details of the spectral shape depend sensitively on the underlying geometry. Because exact molecular data do not exist at present for the tubular J-aggregate, a similar modeling does not appear straightforward and untimely.

If we now consider the effect of the left-handed (*S*)-alcohol, it becomes obvious that the increased CD signal due to the right-handed (*R*)-alcohol does not arise from pure amplification of intrinsic chirality but is the expression of a kind of induced circular dichroism. Figure 1c shows the CD spectra obtained after titration of left-handed alcohol. It is evident that with an increase of the amounts of (*S*)-2-octanol the signature of the CD signal reverses. Reversal occurs at  $R \approx 8$ . For larger mixing ratios, the spectra become mirror images in good approximation. The absorption spectra are not shown because they are identical to those displayed in Figure 1a.

The “reversal experiment” reveals indeed a correlation between the chirality of the alcohol and the aggregates’ CD spectra and verifies our concept outlined in the Introduction, i.e., the control of chirality by chiral long-chain alcohols. The net effect is an increased excess of aggregates with a particular handedness due to alcohol-induced chirality. The molecular mechanism of chirality induction is not known, however. It is thus not possible to draw the sense of handedness of the packing of dye molecules based on CD measurements.

The amplified CD signals reported above were measured immediately (within a few minutes) after the addition of the alcohols to aggregated solutions, which shows that the response is fast despite the enormous geometrical size of the J-aggregates. This finding confirms the trend already observed for the incorporation of other surface-active additives<sup>52,62</sup> and points to a fast and flexible rearrangement of the dye molecules during incorporation of the guest molecules. Nevertheless, it is interesting to look for the size of the alcohol effect when the dye molecules are present as monomers prior to alcohol addition and not organized in a well-ordered supramolecular packing. In recent studies,<sup>18,51,52</sup> it was observed that aggregated solutions prepared from monomers (“alcoholic route”) exhibited markedly enhanced CD signals compared to solutions prepared by dissolving the crystalline dye material directly in the solvent. Therefore, we added a monomeric dye solution of  $C_8O_3$  (dissolved in MeOH) to the solvent, which was  $10^{-2} \text{ M NaOH}$  containing the chiral alcohol (*R*)-2-octanol or (*S*)-2-octanol. The measured absorption and CD spectra are depicted in parts a and b of Figure 2. Because of the presence of alcohol, the absorption spectra exhibit 2-fold split J-bands. The CD spectra show the

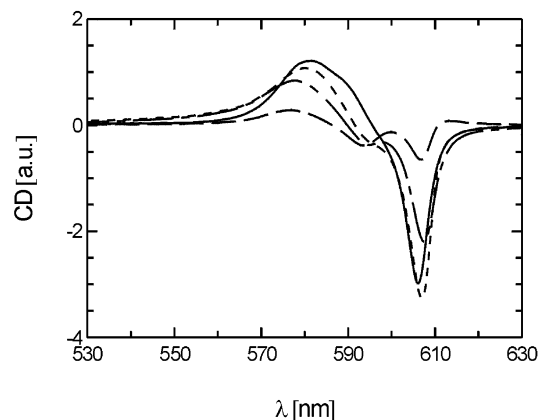


**Figure 2.** Absorption (right ordinate) and CD (left ordinate) spectra of  $6.1 \times 10^{-5}$  M  $\text{C}_8\text{O}_3$  solutions prepared by the “alcoholic route” from monomer solution in MeOH after 1 day of aggregation in  $10^{-2}$  M NaOH containing (a) (*R*)-2-octanol ( $R = 56$ ) and (b) (*S*)-2-octanol ( $R = 50$ ). Total MeOH content was 2.5 wt %. Total EtOH content was 1.8 wt %.

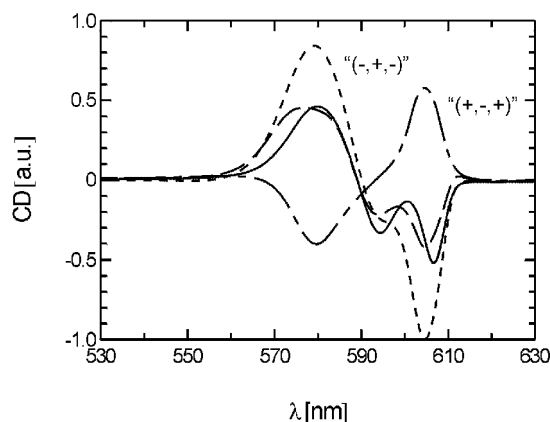
expected signatures for the right- and left-handed alcohols, respectively, but their intensities are increased by a factor of 5 with respect to the spectra of parts b and c of Figure 1. Qualitatively identical spectra, compared with spectra obtained in the presence of (*S*)-2-octanol, were also obtained for (*S*)-2-decanol, with the only difference being that the amount of (*S*)-2-decanol necessary to produce similarly strong CD signals was smaller ( $R = 5.5$ , not shown).

In summary, we have to recognize that the excess of J-aggregates with a particular handedness is markedly enlarged when the chiral alcohols are present in the solvent prior to the beginning of aggregation. Reasons that could give rise to larger CD signals might be a real chiral selection that favors the formation of aggregates with a particular handedness during the aggregation from monomers, a more perfect chiral molecular packing within the aggregates, or an interplay of either effects. The time dependence reflected in the CD spectra discussed in the following section, however, makes a good case against a hypothesis of pure improved order and underlines the importance of additional kinetic effects.

As a rule, longtime studies on alcohol-free solutions have shown increasing CD signals with increasing storage time at ambient temperature, without qualitative changes of the spectra, in particular unchanged  $(+, -, +)$  signature. A different behavior was observed for the great majority of samples containing (*S*)-2-octanol and exhibiting a  $(-, +, -)$  signature. Typical spectra illustrating the occurring changes during storage are given in Figure 3. Here, the mean CD intensity is decreasing with storage time while the shape of the CD spectrum undergoes qualitative changes. After about 1 week of storage, a second negative band peaking at 595 nm emerges and becomes more pronounced at longer times.



**Figure 3.** CD spectra of a (*S*)-2-octanol-containing solution prepared by the “alcoholic route” as a function of storage time after aggregation: 2 days (solid line); 7 days (short dashes); 15 days (dash dot); and 21 days (long dashes).



**Figure 4.** CD spectra of a (*S*)-2-octanol-containing solution (“alcoholic route”) measured 1 day (signature:  $(-, +, -)$ , short dashes) and 7 days (solid line) after preparation. Added is a CD spectrum with  $(+, -, +)$  signature (dash dot) measured in the presence of (*R*)-2-octanol after 1 day of aggregation (weighted with a factor of 0.7). The spectrum indicated by long bars is the linear combination of individual spectra after 1 day of storage that qualitatively reproduces the principal features of the 7-day-old sample.

The principal features of the final spectrum with the split negative band can be reproduced by a superposition of corresponding “pure” spectra of either signature. Figure 4 shows the spectra of two samples prepared by the “alcoholic route” in the presence of (*S*)-2-octanol ( $(-, +, -)$  signature, short bars) and (*R*)-2-octanol ( $(+, -, +)$  signature, dash dot) after 1 day of aggregation. The weighted linear combination (long dashed line) of both spectra indeed reproduces the mean features of the experimental spectrum (solid line) measured after 7 days of storage. It is noted that the “pure” spectra selected here are not perfect mirror images. The  $(-, +, -)$  spectrum already shows a weak shoulder at  $\sim 595$  nm after 1 day of aggregation. Long-term changes of the CD spectra such as those just reported were also observed for solutions containing (*S*)-2-decanol and confirm the observed trend.

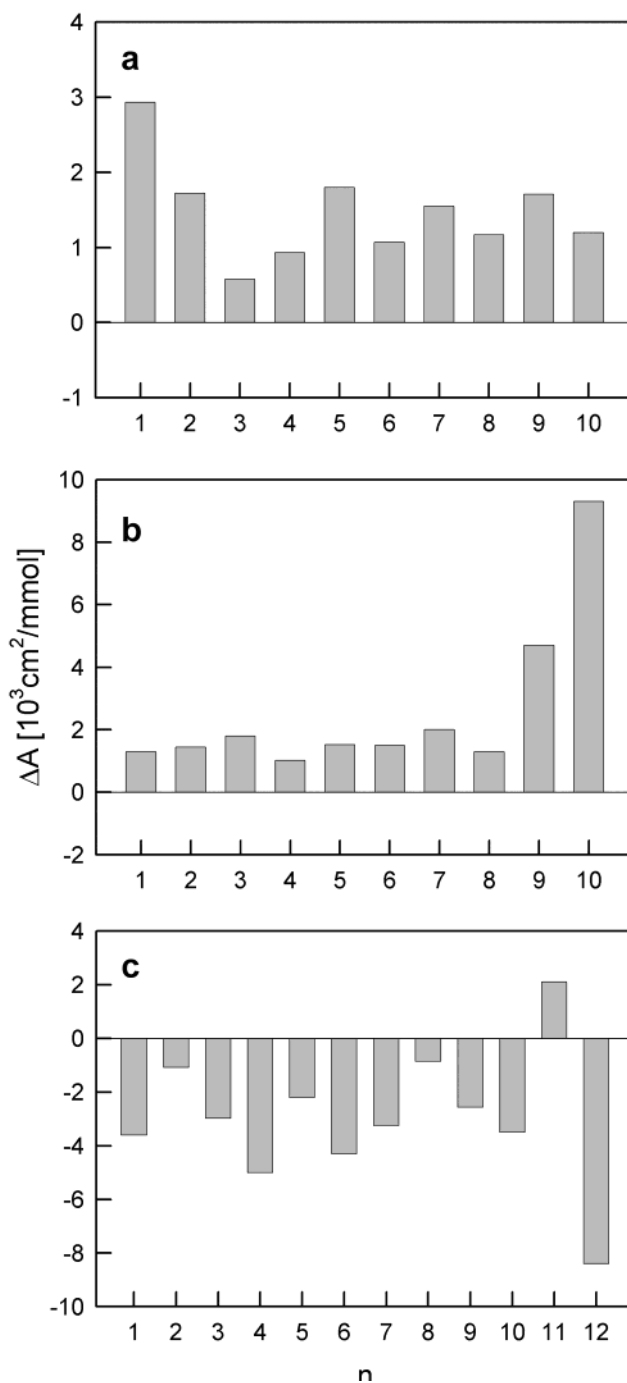
From our recent studies investigating the effect of achiral alcohols upon the  $\text{C}_8\text{O}_3$  J-aggregates, we know that the multiplicity (number of individual tubules) of the ropelike superhelical J-aggregates increases with storage time.<sup>52</sup> A similar thickness growth of the assembled J-aggregates is also expected in the presence of chiral alcohols. The reported long-term changes of CD spectra can be attributed to similar time-consuming molecular and supramolecular rearrangements. The

modeling of spectral changes suggested an increasing contribution from aggregates with  $(+, -, +)$  CD signature; i.e., aggregates with this particular sense of handedness seem to be favored. We thus again arrive at the striking conclusion that the  $C_8O_3$  J-aggregates are characterized by a “natural” sense of handedness. Note that this particular handedness is selected spontaneously in pure solutions<sup>33</sup> as well as in the presence of achiral alcohols.<sup>52</sup> Experiments designed to stimulate the rearrangements by elevating the temperature failed because of the gradual decomposition of the dye.

Because of statistical fluctuations in the process of aggregation, a check of the reproducibility of the chirality effects is a necessary condition for their interpretation. We therefore prepared three series of  $7 \times 10^{-5}$  M dye solutions by the “alcoholic route” in  $10^{-2}$  M NaOH. The first series was prepared from a  $2.31 \times 10^{-3}$  M dye stock solution in methanol. The stock solution was prepared by dissolving  $C_8O_3$  single crystals (pure dye betain) in methanol. This methanolic dye solution was then added dropwise to  $10^{-2}$  M NaOH for aggregation. These mixtures were vigorously shaken by hand so that they became homogeneous, and they were stored without stirring in the dark for 1 day. The other two solutions were obtained from the recrystallized dye material but were otherwise analogously prepared and aggregated without stirring in  $10^{-2}$  M NaOH containing (*R*)- or (*S*)-2-octanol ( $R = 47$ ), respectively. For the octanol-free samples, fresh cuvettes have been taken for each solution. Prior to measurements, the cuvettes were cleaned by sulfuric acid and rinsed by methanol and doubly distilled water.

To quantify the CD strength, we used the difference  $\Delta A$  of the CD-peak maxima at the wavelengths of 605 and 582 nm:  $\Delta A = CD(605) - CD(582)$ . The results are summarized in the histograms of Figure 5. A positive  $\Delta A$  value corresponds to a  $(+, -, +)$  signature, and a negative  $\Delta A$  value corresponds to a  $(-, +, -)$  signature. The octanol-free samples always showed positive  $\Delta A$  values with a magnitude that is characteristic of the “alcoholic route” of sample preparation.<sup>52</sup> This result is consistent with the former finding<sup>33,51,52</sup> that chiral aggregates in a nonracemic mixture are formed for samples where special attention was paid to the purity of the dye material. The  $\Delta A$  values for solutions containing enantiomerically pure alcohols are on average either positive for (*R*)-octanol or negative for (*S*)-2-octanol. The CD strength varies by more than 100% of its value from sample to sample. This variation is larger for the samples with chiral alcohols added (cf. Figure 5). In the case of (*S*)-2-octanol added, even one sample was found with positive  $\Delta A$  value.

The reason for the observed enantiomeric excess is not understood. An influence from stirring as recently reported by Ribó et al.<sup>43</sup> should be excluded because of the absence of stirring during sample preparation. It is generally believed<sup>46,63</sup> that homochirality in chemical processes arises from a small deviation from the achiral initial state coupled to self-amplifying (autocatalytic) processes of structure formation. Recently Singleton and Vo<sup>64</sup> deduced from careful experiments on an autocatalytic reaction (of 2-methylpyrimidine-5-carboxaldehyde with diisopropyl zinc) that chiral impurities present in the reaction solvent at the level of parts per billion (too small to be detected directly) are the source of the developed asymmetry. The nonlinearity of the reaction is the origin of the high sensitivity to minor amounts of chiral additives. Similar processes cannot be excluded for the present system, despite the careful sample preparation. The source of the prospective chiral impurities remains unknown, however.



**Figure 5.** Distribution of CD amplitudes,  $\Delta A$ , for sets of  $7.0 \times 10^{-5}$  M  $C_8O_3$  solutions freshly prepared by the “alcoholic route” (a) in the absence of octanol, (b) in the presence of (*R*)-2-octanol ( $R = 46$ ), (c) and in the presence of (*S*)-2-octanol ( $R = 47$ ) after 1 day of aggregation in  $10^{-2}$  M NaOH.

**Morphology.** Because CD measurements yield information about the chirality on the molecular level, questions arise on how the chirality is related to the morphology of the aggregates. Cryo-TEM has proved to be a powerful tool in characterizing the supramolecular morphology of J-aggregates on a mesoscopic scale.<sup>33,52,62</sup> A cryo-TEM micrograph of a solution containing (*S*)-2-octanol showing the typical ropelike morphology is presented in Figure 6. The morphology resembles that of J-aggregates formed in the presence of different achiral alcohols;<sup>52</sup> e.g., the aggregates are composed of many individual tubular strands of  $\sim 11$  nm thickness that are twisted to form superhelical bundles. The advancing assembly into multiple





**Figure 6.** Cryo-TEM image of  $7.0 \times 10^{-5}$  M aqueous  $C_8O_3$  solutions (containing  $10^{-2}$  M NaOH) prepared by the “alcoholic route” in the presence of (*S*)-2-octanol ( $R = 47$ ) showing a J-aggregate composed of several single tubular strands that are twisted. Bar = 50 nm.

**TABLE 1: Helix Handedness of J-Aggregates**

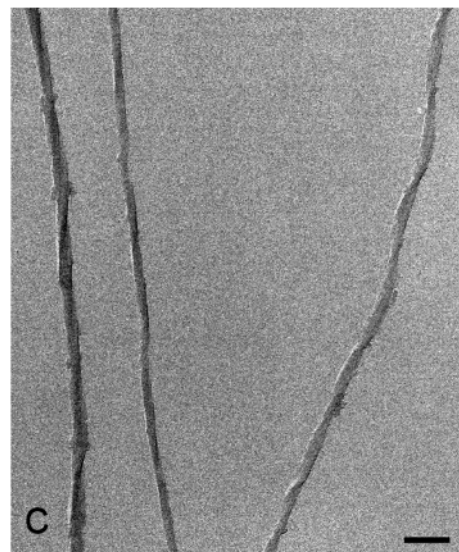
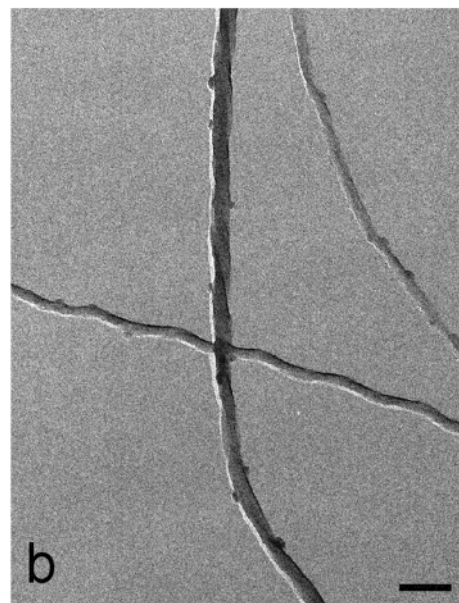
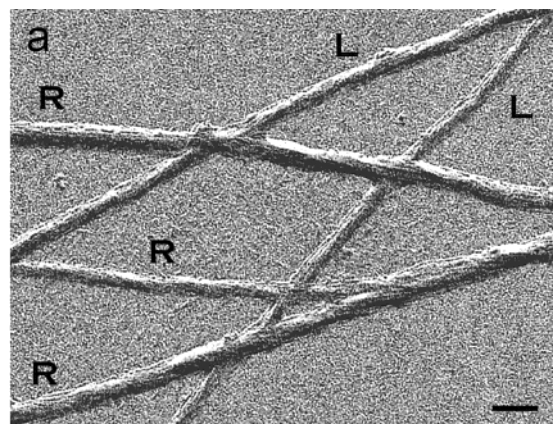
$C_8O_3$ solution	CD strength $\Delta\epsilon$ ( $10^3$ cm <sup>2</sup> /mmol)	number of aggregates	handedness <sup>a</sup> (%)		
			right	left	undefined
alcohol-free <sup>b</sup>	+0.5	225	22	18	60
( <i>R</i> )-2-octanol <sup>c</sup>	+4.1	83	12	58	30
( <i>S</i> )-2-octanol <sup>d</sup>	−5.0	63	48	22	30

<sup>a</sup> The helix handedness was determined from TEM micrographs after deposition and shadowing. <sup>b</sup> Solution:  $3.0 \times 10^{-4}$  M  $C_8O_3$  in  $10^{-2}$  M NaOH, stored for 3 months. <sup>c</sup> Solution:  $6.6 \times 10^{-5}$  M  $C_8O_3$  in  $10^{-2}$  M NaOH/(*R*)-2-octanol,  $R = 49$ , after 3 days. <sup>d</sup> Solution:  $7.0 \times 10^{-5}$  M  $C_8O_3$  in  $10^{-2}$  M NaOH/(*S*)-2-octanol,  $R = 47$ , after 7 days.

bundles upon storage that eventually leads to the precipitation is a general feature of all alcohol-containing solutions and is presumably due to the cooperative interlinking by hydrogen bonds.<sup>52</sup>

Cryo-TEM is often ambiguous for discriminating the sense of handedness. The topographic information needed to discriminate the sense of handedness can be obtained by heavy metal shadowing of deposited aggregates. Typical micrographs are displayed in Figure 7. Aggregates from an alcohol-free solution that was stored for three months are shown in Figure 7a. The long-time storage gave thicker helical bundles, facilitating a straightforward discrimination of the helix handedness. Both senses of handedness are found with roughly equal probability. However, for the larger part of aggregates visible on a micrograph, the proof of a particular handedness was not possible because of the low degree of twisting. The determination of the aggregates' handedness was even more difficult for fresh solutions because of the generally lower multiplicity of bundles. Nevertheless, a clear excess of left-handed aggregates was found for solutions prepared in the presence of (*R*)-2-octanol and an excess of right-handed aggregates in the case in which (*S*)-2-octanol was added. Typical micrographs are shown in parts b and c of Figure 7 for the (*R*)- and (*S*)-2-octanol-containing samples.

To gain better statistics, images were analyzed and several hundreds of aggregates were inspected individually. The results are summarized in Table 1. The majority of aggregates with (+,−,+) CD signature in the presence of (*R*)-2-octanol shows left helix handedness (counterclockwise twist), whereas the majority of aggregates with (−,+,−) CD signature in the presence of the (*S*)-2-octanol reveals a right-handed (clockwise) twist of the bundles. It is emphasized that these results allow us, for the first time, to relate the handedness of the supramolecular morphology to the sign of the optical CD spectra. Since the signature of solutions without any chiral additives is always of the (+,−,+) type, one would expect an excess of left-handed aggregates. On the basis of the statistics of the results in Table 1, we cannot prove this. However, one has to bear in mind that the handedness of the helical twist of about 60% of the aggregates could not be identified and the CD effect was much weaker (by a factor of about  $1/10$ ) than in case of the (*S*)- or (*R*)-2-octanol-induced chirality. Therefore, it seems plausible



**Figure 7.** TEM micrographs of helical  $C_8O_3$  J-aggregates after Pt/C shadowing: (a)  $3.0 \times 10^{-4}$  M  $C_8O_3$  in  $10^{-2}$  M NaOH, with right helix handedness (R, clockwise twist) and left helix handedness (L, counterclockwise twist) indicated (bar = 100 nm); (b)  $6.6 \times 10^{-5}$  M  $C_8O_3$  in  $10^{-2}$  M NaOH/(*R*)-2-octanol,  $R = 49$  (left helix handedness, bar = 200 nm, after 7 days); (c)  $7.0 \times 10^{-5}$  M  $C_8O_3$  in  $10^{-2}$  M NaOH/(*S*)-2-octanol,  $R = 47$  (right helix handedness, bar = 200 nm).

that in such solutions a more balanced distribution of helix handedness is found.

## Summary and Conclusions

The achiral carbocyanine dye  $C_8O_3$  self-aggregates in aqueous solution to form optically active superhelical J-aggregates. A three-phasic CD spectrum with a positive Cotton effect at 568 nm, negative Cotton effect at 582 nm, and positive Cotton effect at 605 nm (+, −, +) is usually found and ascribed to molecular excitons. A set of octanol-free samples prepared by the “alcoholic route” from a monomer solution in methanol without stirring during aggregation showed predominantly CD signals with (+, −, +) signature; i.e., the generation of chirality appears to be enantioselective. This intriguing finding contradicts the expectation that in the chiral symmetry-breaking mechanism both senses of handedness should be realized in equal proportion because of statistical fluctuations. Stirring effects should be excluded. However, despite the careful sample preparation, it cannot be excluded that the observed homochirality arises from minor amounts of chiral impurities of which the source is unknown. The molar circular dichroism increases by a factor of about 100 when the chiral (*R*)-2-octanol is added to an aggregated solution, and the measured signal is further amplified by a factor of about 5 when this alcohol is already present in the solvent prior to the beginning of aggregation. The signature of the CD signal reverses with increasing amounts of the left-handed (*S*)-2-octanol, and for a molar (*S*)-2-octanol/dye ratio of about 20 the spectra become, to good approximation, mirror images. This reversal of signature reveals a correlation between the chirality of the added alcohol and the aggregates’ CD spectra and supports our concept of controlling the chirality of the dye aggregate by incorporation of chiral long-chained alcohols. It is obvious that these alcohols differentiate between the packing chiralities. The net effect is an increased excess of aggregates with a particular sense of handedness. Samples containing (*S*)-2-octanol showed that their “reversed” CD spectra with (−, +, −) signature are not fully stable when stored for longer times at ambient temperature. The spectral changes have been ascribed to an increased contribution from aggregates with the opposite sense of handedness ((+, −, +) CD signature) in the nonracemic mixture. The underlying time-consuming molecular and supramolecular rearrangements obviously favor again the preferred sense of handedness. Heavy metal shadowed samples demonstrate that the majority of aggregates with (+, −, +) CD signature in the presence of the (*R*)-2-octanol shows left helix handedness (counterclockwise twist) and the majority of aggregates with (−, +, −) CD signature in the presence of the (*S*)-2-octanol shows right helix handedness (clockwise twist).

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