Chiral exciton coupling of merocyanine dyes within a well defined hydrogen-bonded assembly

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Edited by George M. Whitesides, Harvard University, Newton, MA, and approved May 12, 2001 (received for review March 16, 2001)

Multichromophoric hydrogen-bonded assemblies 1_3·$(BAR)_6$ are studied that bear a remarkably close resemblance to commelinin, a naturally occurring assembly responsible for an intense blue color of flowers. The incorporated chromophores exhibit a hypsochromic shift in the UV/visible (Vis) absorption maximum ($\Delta\lambda_{max}$ = 14 nm) compared with the free chromophores. In addition, the chiroptical properties of incorporated chromophores can be rationally controlled by changing the supramolecular chirality of the assembly. These properties have been used to study the stability of this type of assembly with UV and CD spectroscopy at concentrations far below the NMR sensitivity threshold (10^{-4} M). The determined $C_{50\%}$ values of 2–3 μM in benzene show the extremely high stability of these *hydrogen-bonded* assemblies.

Most chromophores are susceptible to changes in their optical properties depending on their chemical environment. This principle has been actively exploited for the development of optical sensors (1–3) and devices (4–6). Along these lines, Nature generates a wide variety of different fruit and floral colors from different noncovalent complexes of the natural anthocyanin chromophores (7, 8). These colors cover almost the complete visible spectrum, despite the fact that the structural diversity in the building blocks is surprisingly low. Commelinin is a blue complex in which a total of six anthocyanins and six flavocommelins are pairwise coordinated around two central magnesium cations (Fig. 1*a*) (9). The blue color of commelinin is a direct result of the self-assembly process driven by coordination of the anthocyanins to magnesium and completely disappears on dissociation of the complex. In addition, commelinin is strongly CD active as a result of exciton coupling between the stacked anthocyanin units (Fig. 1*b*). The left-handed screw is a result of chiral induction by sugar moieties present in the components. Numerous synthetic assemblies that contain multiple chromophores in a cyclic (10–13) or polymeric array (14–17) have been reported. However, none of them can match natural assemblies in the sense that they are both molecularly well defined and display (chir)optical properties that are different from the separate components. In this paper we report hydrogen-bonded assemblies that bear a remarkably close resemblance to commelinin (Fig. 1 *c* and *d*). The similarity is not only expressed by the supramolecular structure, but also by a similar change in both the optical and chiroptical properties of the chromophores on formation of the assembly. These spectral changes enable us to study the stability of these assemblies at micromolar concentrations, far below the ^{1}H NMR sensitivity threshold.

The barbiturate BAR has a chromogenic donor-π-acceptor system with a charge-transfer (CT) absorption band in the visible range at λ_{max} = 478 nm in chloroform ($\varepsilon_{478} = 8.2 \times 10^4$ l·mol^{-1}·cm^{-1}). On assembly formation with calix[4]arene dimelamine **1**, BAR is incorporated into a supramolecular structure with composition $\mathbf{1}_3$·$(BAR)_6$ (ref. 18; Fig. 2). The driving force for the assembly process is the cooperative formation of 36 hydrogen bonds between the complementary hydrogen-bond arrays of barbiturates and melamines. On formation of the assembly, the BAR CT absorbance undergoes a hypsochromic shift of 14 nm leading to a new absorption maximum at 465 nm ($\varepsilon_{465} = 5.7 \times 10^4$ l·mol^{-1}·cm^{-1}) (Fig. 3*a*). Evidence for the quantitative formation of assembly $\mathbf{1}_3$·$(BAR)_6$ was obtained from ^{1}H NMR spectroscopy in $CDCl_3$. The spectrum shows the characteristic proton signals at 13.76 and 13.65 ppm for the hydrogen-bonded NH-protons of BAR in assembly $\mathbf{1}_3$·$(BAR)_6$ (19).

Like the anthocyanin units in commelinin, the BAR chromophores in assembly $\mathbf{1}_3$·$(BAR)_6$ reside in a chiral environment as a result of the antiparallel orientation of the melamine fragments. This orientation causes a helical twist (*P* or *M*) of the two stacked rosette motifs (20). Our assignment of *M* and *P* helicity is based on a clockwise (*P*) or counterclockwise (*M*) orientation of the two melamine units of **1** in assembly $\mathbf{1}_3$·$(BAR)_6$. Because both dimelamine **1** and BAR are achiral, assembly $\mathbf{1}_3$·$(BAR)_6$ is formed as a racemic mixture of *P* and *M* enantiomers and therefore is optically inactive (18). However, the supramolecular arrangement of the BAR chromophores can be controlled in a unidirectional manner by using chiral building blocks (21). For example, assembly of the chiral dimelamine (*R*,*R*)-**2** with BAR forms the *M*-helical form [diastereomeric excess (>95%)].[§] Assembly formation was evidenced by ^{1}H NMR spectroscopy (characteristic proton signals at 13.73 and 13.58 ppm) and matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) mass spectrometry after Ag^+-labeling (m/z = 5323.6; calculated for (*M*)-$\mathbf{2}_3$·$(BAR)_6$·Ag^+: 5323.4) (22). Also in this case a shift of the BAR CT absorption band from 478 to 466 nm in $CHCl_3$ was observed on formation of (*M*)-$\mathbf{2}_3$·$(BAR)_6$ (Fig. 4*a*). Furthermore, as a result of the single handedness of this assembly the shift is accompanied by two strong Cotton effects of opposite sign at 481 nm ($\Delta\varepsilon_{481}$ = 606 l·mol^{-1}·cm^{-1}) and at 451 nm ($\Delta\varepsilon_{451}$ = 307 l·mol^{-1}·cm^{-1}). Similarly, assembly of two equivalents of dimelamine (*S*,*S*)-**3** with six equivalents of BAR quantitatively gives the *P* enantiomer of assembly $\mathbf{3}_3$·$(BAR)_6$, as reflected by the mirror image CD (Fig. 4*a*). The bisignate nature of the CD spectra, together with the fact that the intercept with the *x* axis (464 nm) coincides with the maximum in the UV spectrum (466 nm), reveals that the observed CD is predominantly a result of exciton coupling between the pairwise assembled BARs in the assembly (Fig. 1 *c* and *d*). The negative CD observed for the BAR CT absorption band in assembly $\mathbf{3}_3$·$(BAR)_6$ is indicative of an *M* configuration of two BAR chromophores (23). This is in full agreement with our assignment of *P* helicity for assembly $\mathbf{3}_3$·$(BAR)_6$, which is based on the relative orientation of the two melamine fragments of **1**. A gas-phase minimized structure of the assembly clearly

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: CT, charge-transfer; Vis, visible.

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§The d.e. is based on comparison of $\Delta\varepsilon_{298}$ of assembly (*M*)-$\mathbf{2}_3$·$(BAR)_6$ (95 l·mol^{-1}·cm^{-1}) with $\Delta\varepsilon_{298}$ of structurally related quantitatively induced assemblies (95–100 l·mol^{-1}·cm^{-1}) (19, 21). A detailed discussion, including the ^{1}H NMR spectrum, is provided in the supplemental data, which are published on the PNAS web site, www.pnas.org.

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www.pnas.org/cgi/doi/10.1073/pnas.171131398

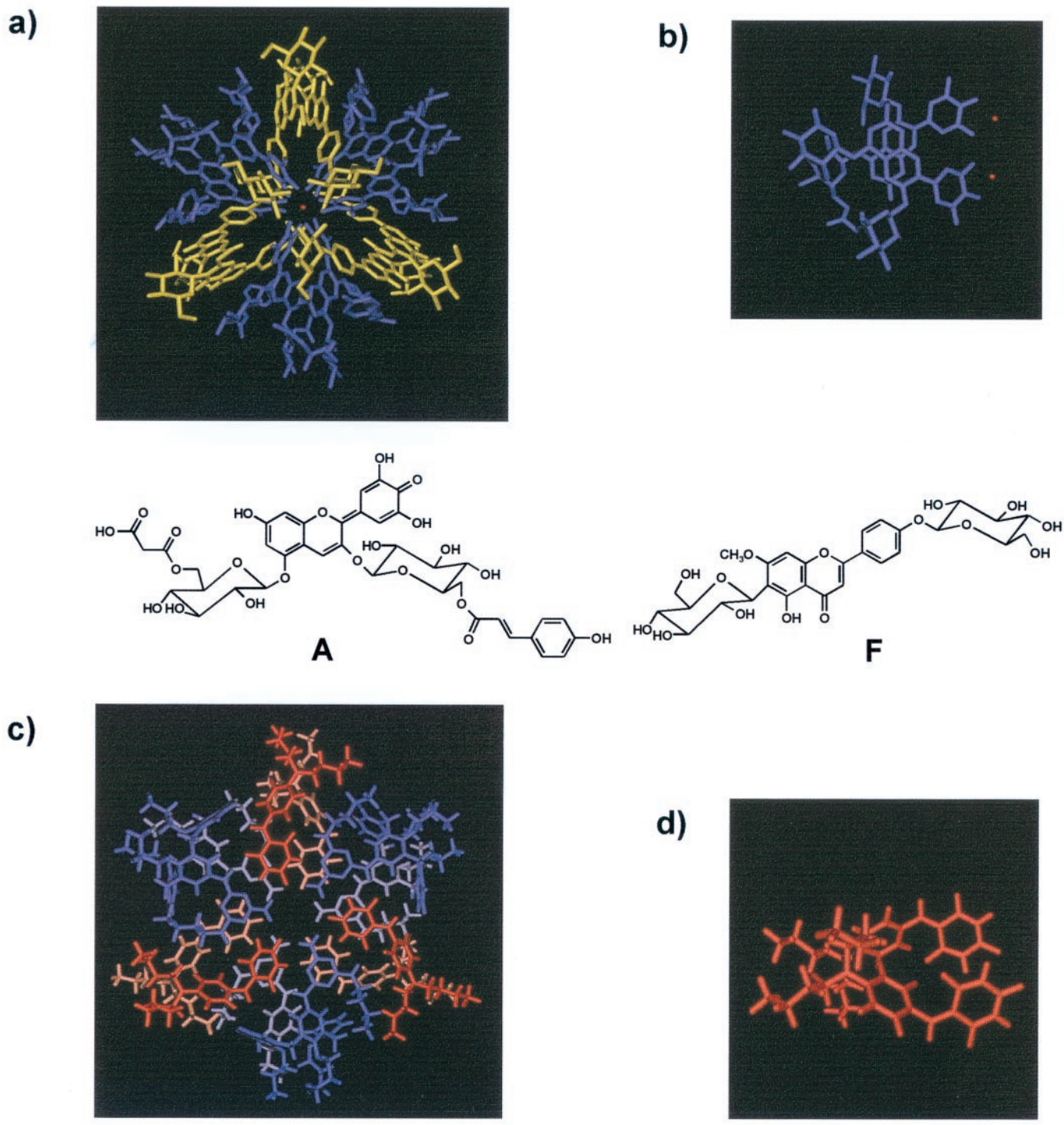

Fig. 1. (*a*) X-ray crystal structure of commelinin composed of six anthocyanin (A, blue) and six flavocommelins (F, yellow) and (*b*) the chiral stack of two anthocyanin chromophores (9). (*c*) Gas-phase minimized structure (CHARMm 24.0) of assembly (*P*)-$\mathbf{1}_3$·$(BAR)_6$ showing (*d*) the pairwise chiral stacks of two complexed BARs. [*a* and *b* reprinted by permission from *Nature* (9), copyright 1992, Macmillan Magazines Ltd. (www.nature.com).]

reveals that two BARs adopt an *M* configuration in an assembly with *P* helicity (Fig. 1 *c* and *d*). Particularly striking is the remarkably close resemblance of the chiral orientation of the six barbiturates in assemblies (*M*)-$\mathbf{2}_3$·$(BAR)_6$ and (*P*)-$\mathbf{3}_3$·$(BAR)_6$ and the six anthocyanin units in commelinin (9).

The characteristic changes of the CT absorption of BAR on formation of assemblies $\mathbf{1}_3$·$(BAR)_6$, (*M*)-$\mathbf{2}_3$·$(BAR)_6$, and (*P*)-$\mathbf{3}_3$·$(BAR)_6$ provide a tool to study the stability of these assemblies with UV/visible (Vis) and CD spectroscopy at concentrations far below the NMR sensitivity threshold (<0.1 mM). The UV/Vis dilution study of assembly $\mathbf{1}_3$·$(BAR)_6$ in $CHCl_3$ is shown in Fig. 3*a*. On dilution of a 1-mM solution of $\mathbf{1}_3$·$(BAR)_6$, the equilibrium of the hydrogen-bonded assembly shifts to the free components and as a result the absorption maximum shifts from 465 nm for complexed BAR to 479 nm for free BAR. The presence of an isosbestic point at 464 nm and spectral changes

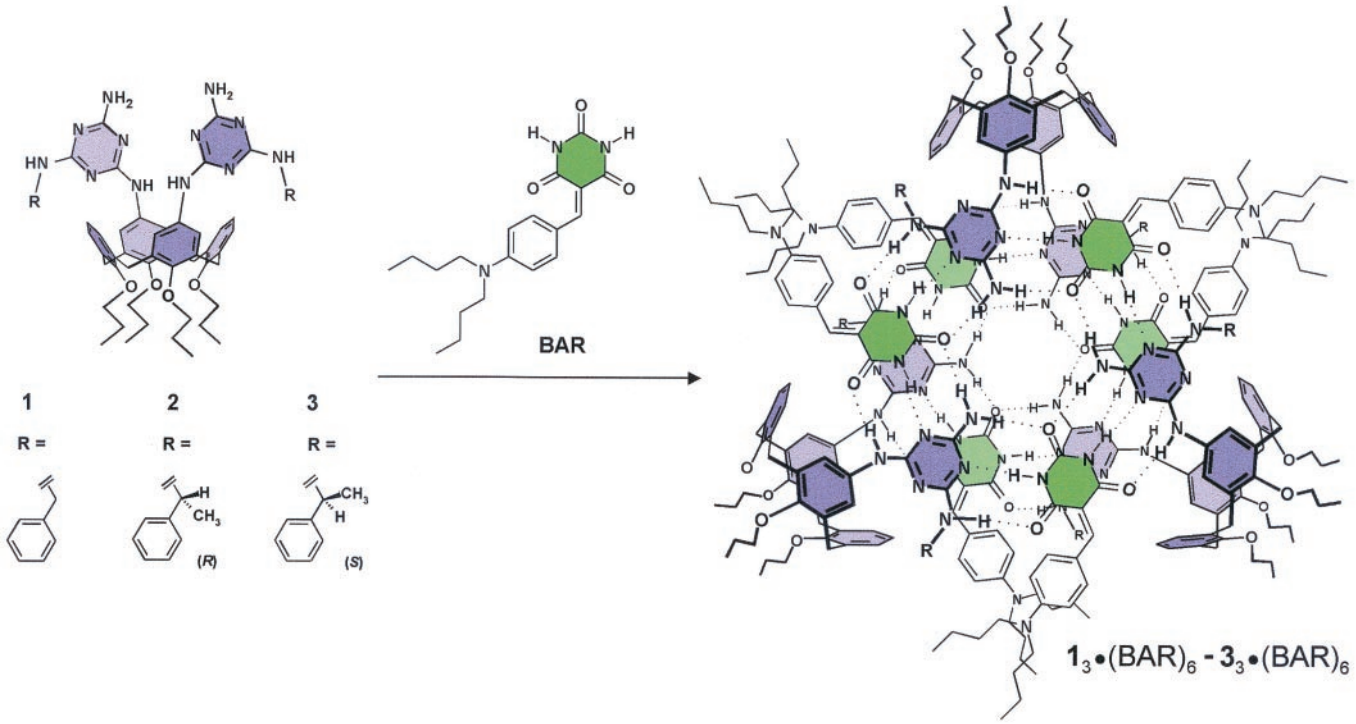

Fig. 2. Schematic representations and molecular structure of the nine-component hydrogen-bonded assemblies $\mathbf{1}_3$·(BAR)$_6$ – $\mathbf{3}_3$·(BAR)$_6$ and their individual components **1**–**3** and BAR.

within a fairly narrow concentration range suggest a highly cooperative two-state equilibrium between two species—i.e., the free chromophores and the chromophores incorporated in assembly $\mathbf{1}_3$·(BAR)$_6$. A $C_{50\%}$ value—i.e., the concentration at which 50% of BAR is incorporated—of 25 μM in chloroform was obtained from a plot of the mole fraction complexed BAR as a function of the concentration (Fig. 3*b*). From a UV/Vis dilution study of assembly (*M*)-$\mathbf{2}_3$·(BAR)$_6$ in $CHCl_3$, a $C_{50\%}$ value of 90 μM was determined (Fig. 4*b*). Supporting evidence that the UV/Vis shift of the CT absorption band of BAR in the dilution studies is related to dissociation of the assembly was obtained by concentration-dependent CD spectroscopy of assembly (*M*)-$\mathbf{2}_3$·(BAR)$_6$ in $CHCl_3$. On dissociation of BAR the CD of the BAR CT absorption band decreases in intensity, because the chromophore no longer senses the chiral environment of the assembly. From a plot of $\Delta\varepsilon_{480}$ versus the concentration [**2**]/3 a $C_{50\%}$ value of 80 μM was determined, which is in close agreement with the value obtained from the UV/Vis dilution studies (Fig. 4*c*).

The utility of UV and CD as analytical tools for the characterization of double rosette assemblies is even better illustrated when studying assemblies $\mathbf{1}_3$·(BAR)$_6$ and (*M*)-$\mathbf{2}_3$·(BAR)$_6$ in benzene. Much to our surprise, we observed precipitation on mixing either **1** or **2** with BAR (1 mM in assembly) in benzene-d_6.

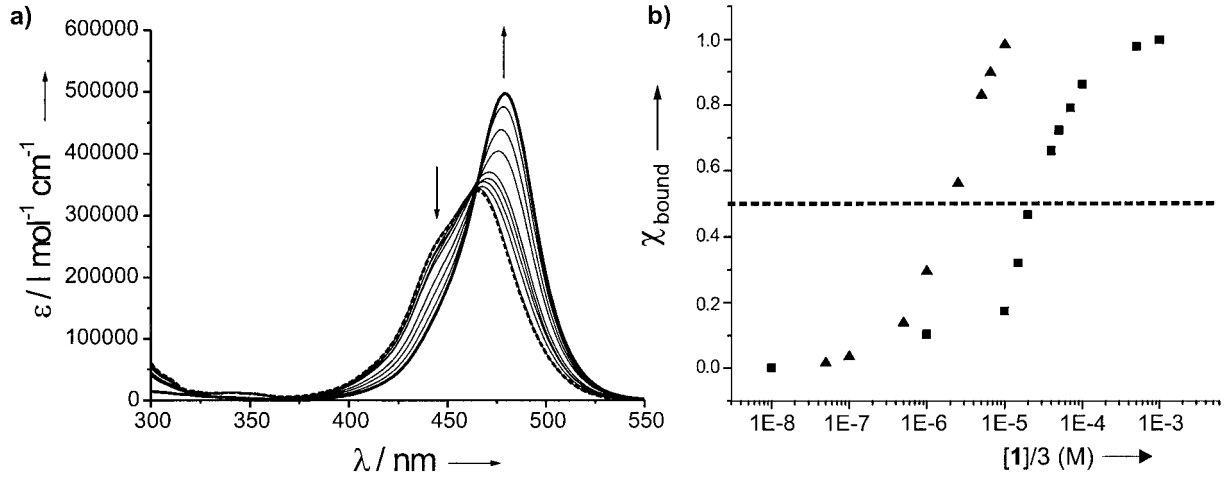

Fig. 3. (*a*) UV/Vis dilution study of assembly $\mathbf{1}_3$·(BAR)$_6$ in chloroform (10^{-3} to10^{-6} M) showing the shift of the BAR CT absorption band. Arrows indicate changes on dilution [dashed line, complexed BAR; solid bold line, free BAR (six equivalent)]. (*b*) Plot of the mole fraction of complexed BAR (χ_{bound}) vs. [**1**]/3 in chloroform (■) and benzene (▲).

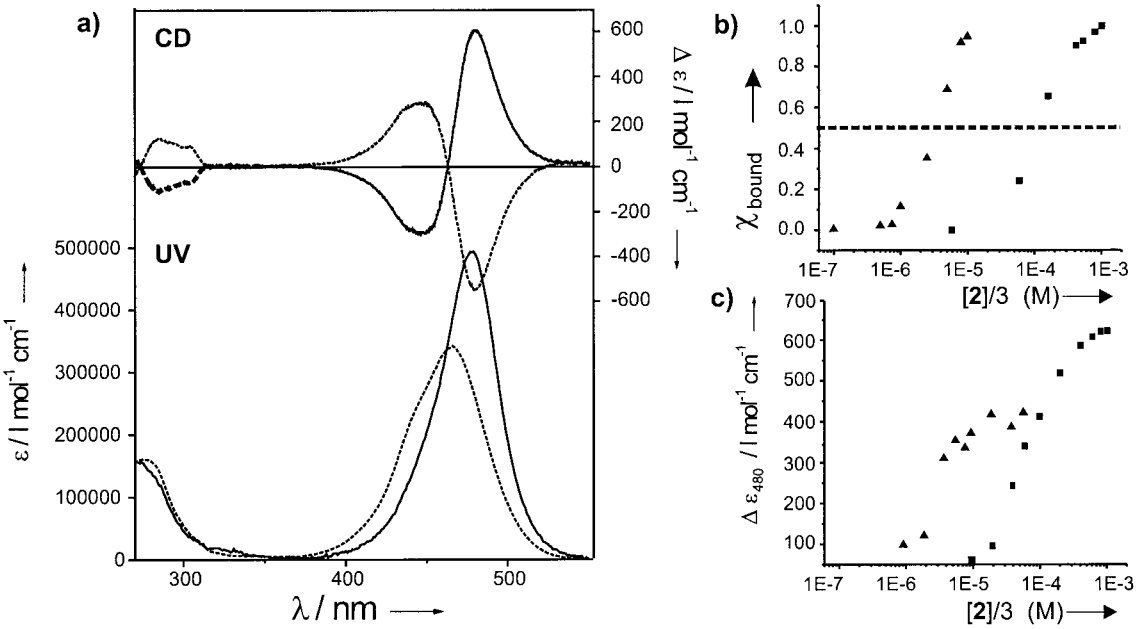

Fig. 4. (*a*) CD spectra of assemblies (*M*)-$\mathbf{2}_3$·(BAR)$_6$ (solid line) and (*P*)-$\mathbf{3}_3$·(BAR)$_6$ (dashed line) and UV spectra of free BAR (six equivalent, solid line) and assembly (*M*)-$\mathbf{2}_3$·(BAR)$_6$ (dashed line). All spectra were recorded in chloroform. (*b*) Plot of the mole fraction of complexed BAR vs. [**2**]/3 in chloroform (■) and benzene (▲). (*c*) Plot of $\Delta\varepsilon_{480}$ vs. [**2**]/3 in chloroform (■) and benzene (▲).

Moreover, the ^{1}H NMR spectra recorded of the saturated solutions did not show any of the diagnostic proton signals at low field (13–16 ppm region) for double rosette assembly formation. However, studying the formation of assemblies $\mathbf{1}_3$·(BAR)$_6$ and (*M*)-$\mathbf{2}_3$·(BAR)$_6$ at much lower concentrations (0.1–100 μM) by UV/Vis and CD spectroscopy revealed that the assemblies had actually formed. UV/Vis dilution studies clearly show spectral changes for the BAR CT-absorption band on dissociation, both for assembly $\mathbf{1}_3$·(BAR)$_6$ and (*M*)-$\mathbf{2}_3$·(BAR)$_6$. Furthermore, for assembly (*M*)-$\mathbf{2}_3$·(BAR)$_6$ a strong CD was observed that decreased in intensity on dilution (Fig. 4*c*). In fact, the assemblies have a strongly increased stability in benzene ($C_{50\%}$ values of 2 and 3 μM, respectively) in comparison to chloroform (Figs. 3*b* and 4*b*). However, their solubility (60–80 μM) is too low to be detected by ^{1}H NMR spectroscopy.

In summary, we have shown that six merocyanine chromophores can be arranged in a chiral screw sense within a well defined supramolecular architecture. The screw sense is rationally controlled by the chiral configuration of the complementary dimelamine building blocks. As a result of dipole–dipole interactions between the chromophores, excitonic coupling is observed giving rise to strong Cotton effects. A practical application of the (chir)optical properties of these assemblies is the possibility to study assembly formation at low concentrations down to 10^{-6} M.

We are grateful to Mr. R. Fokkens and Prof. N. M. M. Nibbering for the matrix-assisted laser desorption ionization time-of-flight (MALDI-TOF) MS measurements. L.J.P. thanks the Council for Chemical Sciences of the Netherlands Organization for Scientific Research (CW-NWO) for financial support. F.W. is grateful to Deutsche Forschungsgemeinschaft (fellowship and research grant Wu 317/1-2) and to Fonds der Chemischen Industrie (Liebig fellowship).

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